

Acta Medica Scandinavica

Redactores

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Editor

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WHOLE BODY AUTORADIOGRAPHY AND FLUOROGRAPHY OF TWO TETRACYCLINE COMPOUNDS IN TUMOUR BEARING MICE

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Abstract The radioactivity and fluorescence distribution pictures of tritium labelled tetracycline in some tumour bearing mice were investigated by means of autoradiography and fluorescence technique applied to whole body sections. In some tissues including blood, bone marrow, lung, heart muscle, red pulp and marginal zone of the spleen, thyroid, brown fat, stomach (contents) and kidney fluorescence was weaker than would be expected from the concentration of the drug. The reasons for this are discussed. A high tissue content of calcium seems to enhance the fluorescence more than can be accounted for only by the concentration. In the von Kossa positive parts of an osteosarcoma fluorescence and to a less extent radioactivity was strong and persistent. In the two von Kossa negative soft tumours fluorescence as well as radioactivity was rather weak.

After the administration of demethylchlorotetracycline to mice bearing ten different kinds of tumours, persisting fluorescence after 24 h was seen only in three osteosarcomas and one mammary carcinoma and was confined to von Kossa positive areas.

Our results favour the view that tetracyclines are retained in tumour tissue with an increased calcium content which is probably less important in increasing the real concentration of the drugs than in enhancing their fluorescence.

In an earlier paper (7) the fluorescence properties of different tetracyclines as well as their distribution in normal tissues were investigated by means of fluorescence technique applied to whole body sections of mice. The distribution pattern was compared to that obtained by autoradiography of tritium labelled tetracycline (H^3 TC). A striking similarity between autoradiogram and fluorogram was seen although some differences were also noted. However the two techniques were used on different animals which to some extent may explain some of these differences.

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In the literature there are many reports on the affinity of tetracyclines for tumour tissue. Most of the studies have been made with fluorescence technique using different tetracycline compounds though in a few investigations radioactive tetracyclines were used. The only investigation comparing the two techniques is that of Machado et al (21) who found the correlation between radioactivity (estimated by means of tritium assay) and fluorescence intensity of H^3 TC to be surprisingly low.

In order to further investigate the validity of fluorescence as a reflection of the real concentration of tetracycline (TC) in tumours and normal tissues it was thought to be of interest to compare autoradiograms and fluorograms from the same tumour bearing animal and even from identical sections. Further to gain some more information on the possible persistence of tetracyclines in tumour tissue a number of mice bearing different kinds of tumours were investigated for fluorescence at 24 h after the administration of the tetracycline compound demethylchlorotetracycline (DMCTC).

MATERIAL AND METHODS

H^3 TC was used the specific radioactivity as given by the manufacturer being 2 mCi/mg (New England Nuclear Corp. Boston, Mass.). To obtain optimal fluorescence non-labelled TC was added as carrier (Achromycin E). The final product had a specific radioactivity of 0.6 mCi/mg. It was administered intravenously as a single dose of 4 mg/kg of body weight to four mice bearing a radiostrontium induced osteosarcoma (osteoblastic medium) transplanted through four generations (osteosarcomas supplied by Dr A. Nilsson, Research Institute of National Defence, Sönderbyberg, Sweden). The animals were sacrificed at 10 min, 80 min, 30 min and 74 h

Table 1 Fluorescence of demethylchlortetracycline in different tumours of mice at 5 and 24 h after injection

Type of tumour	Induction	Re-trans-plantations	Administra-tion	Fluorescence (5 h)	Fluorescence (24 h)
Fibroblastic osteosarcoma	Radiation	Few	I v	3+	1+
Fibroblastic osteoblastic sarcoma	Radiation	Few	I p	3+	2+
Osteoblastic sarcoma (intense bone formation)	Radiation	Few	I v	No obs	4+
Thymoma SES 3 HT	Polyoma virus	Several	I v	3+	0
Thymoma SECBT	Polyoma virus	Several	I v	2+	0
Mammary carcinoma SECR	Polyoma virus	Several	I v ^a	(+)	0
Mammary carcinoma SECX	Polyoma virus	Several	I p	No obs	3+ ^b
Sarcoma SEC 2 H	Polyoma virus	Several	I p	2+	0
Fibrosarcoma	Methylchol anthrene	None	I v	2+	0
Primary not identified	Polyoma virus	None	I v	No obs	0

^a The 100 mg of demethylchlortetracycline were divided into four doses

^b Fluorescence was observed only in several small spots in the tumour

after injection. For comparison with soft non bone forming tumour tissue one mouse with a thymoma (SES-3M) and another mouse with a sarcoma (SES-C-7) (soft tumours supplied by Dr O Sjogren Dept of Tumour Biology Karolinska Institutet Stockholm, Sweden) both induced by means of polyoma virus and transplanted through several generations were sacrificed at 20 min after injection. One osteosarcoma bearing animal served as a control to study the autofluorescence of the tissues.

To investigate the persistence of fluorescence in experimental tumours another series of mice were used bearing ten different tumours comprising three radio strontium-induced osteosarcomas with different degree of bone formation six soft tumours induced by means of polyoma virus and one soft tumour induced by means of methylcholanthrene (Table 1). In this study DMCTC (Ledermycin[®]) was used since the fluorescence of this compound has proved to be less pH-dependent and possibly to have a more suitable colour (orange yellow) than others of the tetracycline group (7). It was administered intravenously or intraperitoneally in a dose of 100 mg/kg of body weight, and the mice were killed at 5 h and 24 h after injection.

Tape sectioning technique

Killing of the mice was accomplished by immersion into a mixture of solid CO₂ and acetone (-75°C) after ether anaesthesia. At -10°C sagittal sections through the animals were cut according to the tape sectioning technique described by Ullberg (for references see (7)) the thickness of the sections being 60 μ which has proved suitable for fluorescence purp.

Fluorescence autoradiography and staining techniques

After drying in the freeze room the sections were photographed under UV light using the method described in a previous communication (7) (light source Philips HPW 155 W photographic registration obtained on Kodachrome X daylight colour film through a Kodak Wratten 2 E filter). As reported it is possible to convert colour photographs into black and white paper prints by rephotographing them through a monochromatic filter mainly transmittable for yellow light. This procedure however has proved incapable of excluding all non yellow light and thus in the present work the estimations of fluorescence intensity were always made from the original colour photographs. The photographed sections from the animals given H³TC were used for autoradiography according to the method of Ullberg (exposure obtained by apposition against Ilford G5 nuclear emulsion plates time of exposure four months). Finally the sections were stained for calcium by means of the von Kossa technique.

RESULTS

Comparison between the fluorescence and autoradiographic distribution pictures of tritium labelled tetracycline

Fluorograms and autoradiograms of mice killed at 20 min 80 min and 24 h after injection of H³TC are shown in Figs 5 and 6. In many organs there is a close correlation between fluores

Sublingual gland

Submaxillary gland

Sublingual gland

Submaxillary gland

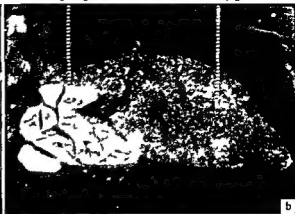
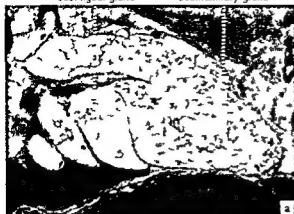


Fig 1 (a) Detail autoradiogram showing higher uptake of H^3 TC in submaxillary gland than in sublingual gland at 0 min after injection (b) Detail autoradiogram showing higher uptake of H^3 TC in sublingual gland than in

submaxillary gland at 80 min after injection. The concentration in the sublingual gland is also higher than at 0 min.

cence and radioactivity. However, in several tissues little or no fluorescence is observed in spite of the fact that H^3 TC is obviously present as judged from the autoradiograms. These tissues include the blood, bone marrow, lung, heart, muscle, spleen (red pulp and marginal zone), thyroid, brown fat, stomach (contents) and kidney.

At 20 min and 80 min the liver shows maximal radioactivity as also do small areas in bone and teeth, although the fluorescence of the liver is not so strong as that of the latter organs. Thus, in bone and teeth the fluorescence of H^3 TC is

stronger than would be expected from its concentration as judged from the autoradiograms. This is still more so in the mineralizing parts of the osteosarcoma where maximal and persisting fluorescence is seen although the radioactivity of this tissue is lower than for example in the liver. The only soft organ behaving in a similar way is the sublingual gland which at 20 min exhibits as strong fluorescence as the submaxillary gland in spite of much lower radioactivity.

Another finding regarding the salivary glands is that at 80 min the sublingual gland has the higher radioactivity of the two glands and also a higher radioactivity than at 20 min (Fig 1).

Some organs may need some further notice. The parathyroid gland accumulates H^3 TC to a remarkable extent at 20 min (Fig 2) and 80 min but is quite devoid of fluorescence and radioactivity at 24 h. The brain, testis and thyroid colloid never show fluorescence or radioactivity. Fat and fibrous cartilage (intervertebral discs) never fluoresce but initially show slight radioactivity. In the remaining soft organs fluorescence fairly well parallels radioactivity with various degrees of initial uptake and due decay at 320 min and 24 h. The bone and teeth still exhibit persisting maximal intensity of fluorescence and radioactivity 24 h after injection. Somewhat lower and less persisting fluorescence and radioactivity is seen in hyaline cartilage (somewhat higher in tracheal than in articular cartilage).

Thyroid Parathyroid gland



Fig 2 Detail autoradiogram of thyroid and parathyroid at 0 min after injection of H^3 TC. Note high uptake in parathyroid gland.

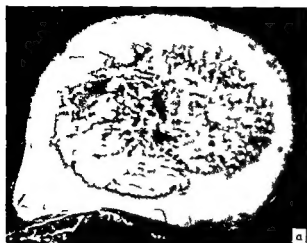


Fig 3 (a) Autoradiogram showing the distribution of H^3 TC in a bone forming tumour (osteoblastic medium) at 80 min after injection (b) Corresponding section of



the autoradiogram in Fig 3a stained according to the von Kossa technique. The uptake of H^3 TC in Fig 3a corresponds to areas of high calcium content.

In the osteosarcoma persisting very strong fluorescence and rather high radioactivity is seen in the mineralized parts (as judged from the von Kossa stained section) as mentioned above (Fig 3). The haemorrhagic parts of the tumour do not contain H^3 TC whereas the peripheral parts with out signs of bleeding and with little or no calcification show a rather high uptake at 20 min but lose it at approximately the same rate as most organs. In the soft tumours which are von Kossa negative (Fig 4) a concentration of H^3 TC is seen which approximately amounts to that of the lymphatic organs but by no means reaches that of the skeleton or the excreting organs (Fig 7). Only one mouse bearing each kind of soft tumour was available and was sacrificed at 80 min after

injection and thus an estimation of the possible persistence of H^3 TC at 320 min and 24 h could not be made. The intensity of radioactivity slightly exceeds that of fluorescence in both soft tumours.

The persistence of demethylchlortetracycline induced fluorescence in different tumours of mice

The types of tumours are listed in Table I. The fluorescence intensity has been divided into six degrees (4+ = very strong, 3+ = strong, 2+ = moderate, 1+ = weak, (+) = doubtful, 0 = no fluorescence). At 5 h after injection all tumours observed show fluorescence of varying intensity. At 24 h the osteosarcoma with intense bone

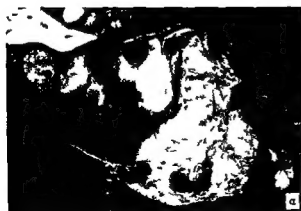


Fig 4 (a) Autoradiographic distribution of H^3 TC in a soft tumour (thymoma) at 80 min after injection (a) and the



corresponding von Kossa stained section (b). Note rather low uptake of H^3 TC and absence of calcium.

formation exhibits strong and homogenous fluorescence the other osteosarcomas also show considerable fluorescence but only in rather small spots. One mammary carcinoma also shows spotted fluorescence at 24 h the other soft tumours being completely devoid of fluorescence. The four tumours with fluorescence at 24 h (that is three osteosarcomas and one mammary carcinoma) are the only ones to show positive von Kossa stain and the fluorescing parts and the von Kossa positive parts of the tumours coincide exactly (Fig. 8). This may indicate the presence of calcium in those areas where persisting fluorescence is seen.

DISCUSSION

Tissue fluorescence and radioactivity of tritium labelled tetracycline

In many tissues good correlation is seen between the fluorescence and radioactivity of H^3 TC (Figs 5, 6 and 7).

However in several tissues or organs fluorescence is weaker than would be expected from the concentration of the compound. Thus in the blood no fluorescence is seen which is in keeping with earlier observations that blood pigment quenches the fluorescence not only of tetracyclines but also of other compounds. The richness of blood may explain why very little or no fluorescence is exhibited also by the bone marrow, lungs, red pulp and marginal zone of the spleen and heart muscle (in contrast to skeletal muscle). In some of these tissues fluorescence has been demonstrated by other authors (e.g. 8) by means of ultraviolet microscopy which obviously makes it possible to observe fluorescing tissue between the erythrocytes. In the brown fat the "quenching theory" may possibly also hold true since the cells of this tissue likewise contain a pigment though of unknown composition. The thyroid gland never shows gross fluorescence it also seems to be quite devoid of radioactivity but at high magnification it can be seen that it is only the colloid which does not contain H^3 TC the narrow cellular areas showing weak radioactivity (Fig. 2). It seems possible that these areas are too small to exhibit gross fluorescence and that they perhaps would fluoresce under the UV microscope. The stomach contents show rather strong radioactivity but no fluorescence.

An explanation would be their acidity since the fluorescence of tetracyclines diminishes or disappears at an acid pH. This factor may be of importance also in the urine the pH of which is about 5-6 in mice fed a standard diet. The relatively weak fluorescence of the kidney may be due partly to the acidity of the urine and partly to the high blood content of this organ.

The mineralized parts of the osteosarcoma and to a less extent bone and teeth exhibit stronger fluorescence than would be expected from their concentration of H^3 TC. The only soft organ behaving in a similar way is the sublingual gland in which at 20 min strong fluorescence but rather low radioactivity is seen it is also more stained by the von Kossa method than almost all other soft tissues which may indicate a higher calcium content. However no persisting fluorescence at 24 h is seen in this organ in contrast to the above mentioned hard tissues.

The sublingual gland which is a mucous salivary gland is also a unique organ in showing higher radioactivity at 80 min than at 20 min.

The only report so far of an accumulation of a tetracycline compound in the parathyroid gland seems to be that of Andre in 1956 who found a high uptake of H^3 TC at 30 min after injection (2). Our autoradiograms confirm this finding showing a high concentration at 20 min (Fig. 2) and still rather high at 80 min. The fluorescence seen on the colour photographs appears somewhat weaker probably because of the very small size of the organ. The parathyroid was not present on the sections from the 320 min mouse but at 24 h it is completely devoid of fluorescence and radioactivity. Anyhow the fact remains that H^3 TC is strongly taken up by an organ known to regulate calcium metabolism.

The affinity of tetracycline compounds for tumour tissue

A rather abundant literature on this subject has appeared during the last years. The majority of the investigations have been performed with the aid of the macroscopic or microscopic fluorescence of tetracyclines. A much discussed question has been to what components of the tumour tissue the drugs are bound. There seem to be three main opinions in this respect some investigators maintain that the fluorescence is seen only in macrophages and cellular debris (1, 26).

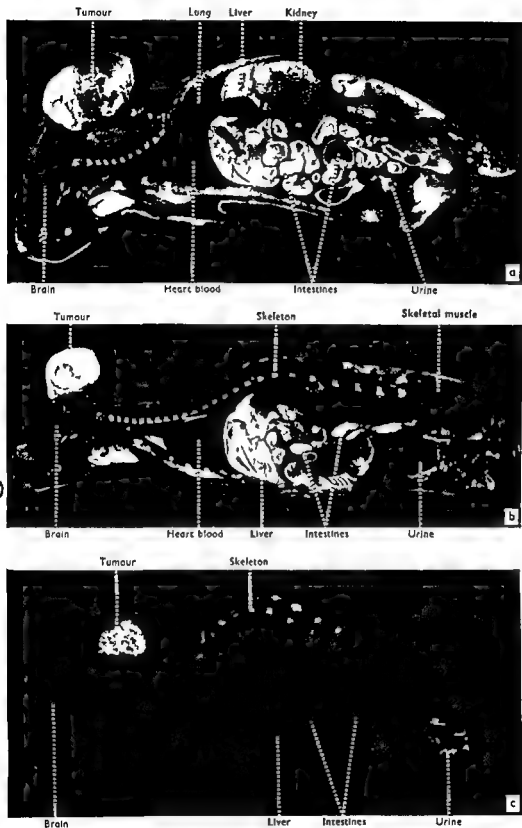


Fig 5 Whole body autoradiographs of mice with a bone forming tumour (osteoblasts medium) killed at 70 min (a) 80 min (b) and 140 min (c) after the injection of ^{45}Ca TC. See text for details

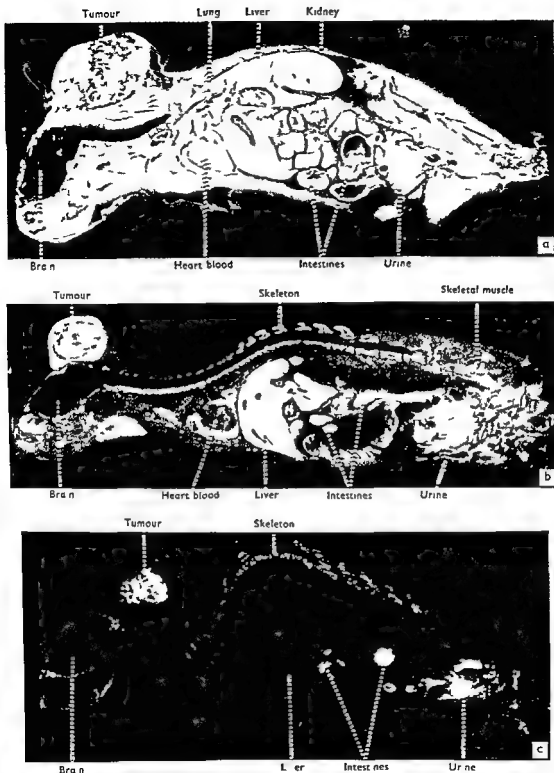


Fig. 6. Fluorescence of H TC exhibited by the sections from which the autoradiograms in Fig. 5 were obtained (a = 0 min, b = 80 min and c = 4 h after injection). The organs with the strongest fluorescence have a whitish

yellow colour. The spontaneously red fluorescing organ behind the eye is the Harderian gland of rodents. See text for details.

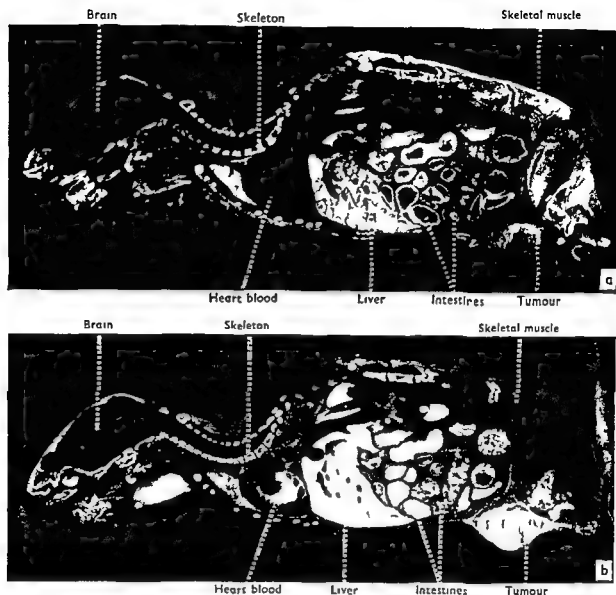


Fig 7 Fluorogram (a) and autoradiogram (b) from a mouse with a soft tumour (sarcoma) at 80 min after the

injection of H 125. Note rather weak fluorescence and low radioactivity in the tumour.

others that it is found within live tumour cells (e.g. 4) and still others that tetracyclines are accumulated or at least retained only in tumour cells undergoing necrosis in which the presence of calcium is regarded by some to play a part.

Findings supporting the latter theory have been reported by many authors. Donsky (10) macroscopically estimated the fluorescence to be located in necrotic or granulomatous areas of skin carcinomas. Barlow et al. (5) found fluorescence in calcified incrustations of bladder carcinomas and only rarely within the tumour tissue itself. Engel

bart et al. (13) observed lasting fluorescence only in grossly necrotic areas of 33 tumours of mice, rats, hamsters and rabbits. Machado et al. (21) found persisting fluorescence in sarcomas 37 and Ehrlich solid tumours of mice. Fluorescence was confined to tumour cells in different stages of degeneration while none was seen in well preserved tumour cells in zones of advanced necrosis with indistinct cell morphology or in the stroma. Very similar results have been obtained by Tapp et al. (29) in squamous skin carcinomas of rabbits and in ovarian tumours, mammary tumours



Fig 8 Persisting fluorescence of DMCTC (a) in the von Kossa positive areas (b) of a mammary carcinoma at 4 h after injection.



and stem cell leukemias of rats. The latter authors further induced necroses in initially non necrotic non fluorescent mammary carcinomas by ligation of the arteries and afterwards found fluorescence in cells undergoing necrosis but not in live tumour cells nor in dead structureless areas devoid of circulation. They also succeeded in staining necrotic parts of tumours with TC in vitro (30) and found the uptake to be highest in areas showing a positive von Kossa stain although some what weaker fluorescence was seen also in the von Kossa negative areas in both these types of necrotic tissue fluorescence could be prevented by pretreatment with Na EDTA.

Somewhat puzzling results in this respect are reported by Riley et al (25). They agree that TC is bound to calcifications within tumour cells but have been unable to find any signs of necrosis even with the aid of electron microscopy. This is at variance with earlier findings that the calcium content of live tumour tissue is generally lower than normal (9, 28) but rises to abnormally high levels when necrosis begins (28).

A considerable amount of evidence however has been put forward in recent years that tetracyclines are fixed in necrotic cells of many kinds irrespective of the type of cell or of what lies behind the necrosis. Malek et al have studied the fixation of tetracyclines (mostly chlortetracycline

CTC) in experimentally induced ischaemic foci of canine myocardium (22). This fixed CTC is said to differ from CTC harbouring in non damaged tissue in having a stronger yellow colour and being resistant to boiling alkali and formol

fixation. The ischaemic foci have been found to be von Kossa positive and to take up increased amounts of radiocalcium (22). Tapp, Carroll and Kovacs (30) have demonstrated the retention of TC in necrotic cells of different tissues in addition to the experimental tumours mentioned above whether the origin of the necrosis is ischaemia or various toxic drugs. They found the fluorescence to be strongest in von Kossa positive areas and since it is known that necrotic parts of the liver and of tumours (28) contain increased amounts of calcium they conclude that tetracyclines may be retained in necrotic cells through binding to calcium. The fact that also von Kossa negative necrotic tissue may exhibit some fluorescence might mean that tetracycline fluorescence is more sensitive for demonstration of calcium than the von Kossa technique.

Though unproved a similar mechanism might possibly lie behind the fixation of tetracyclines in an additional number of pathologically altered tissues, including bronchopneumonic and pyelonephritic foci, inflammatory necroses, subcutaneous granulomas and cutaneous microabscesses, skin ulcers, stomach ulcers, necroses of kidneys and livers, necrotic areas after liver resection, torn skeletal muscle, burns, cadmium induced degenerative changes of the testes and cells damaged by virus in vitro.

The calcium hypothesis gains further support from the fact that tetracyclines are chelating agents (15) forming complexes with certain metal ions such as calcium and zinc. This could account for the fixation of tetracyclines in virtually all

tissues normal or pathological where gross calcification is known to occur (e.g. 1, 11, 13, 16, 27). Moreover it has been shown that tetracyclines form precipitates with beta lipoproteins only in the presence of calcium (6) and are able to bind macromolecules (DNA and albumin) through the mediation of calcium and zinc (18).

To these findings can be added the work of Finerman and Milch (15) who considered the persistence of tetracycline fluorescence in biological tissues to be directly related to the calcium content of the tissue, probably by the interaction with calcium ions in "hydroxyapatite seeded nucleation sites" on collagen fibrils. Loo et al. (20) in 1957 without taking calcium into account maintained that in sarcoma 37 of mice TC is bound by forming a complex with a peptide it possibly could be suggested then that this complex would be mediated by calcium.

It is also to be noted that other compounds than tetracyclines are known to localize in tumours and that some of these also accumulate in bone for example some of the porphyrins (3, 19) and the dye Alizarin red S (21).

Our own experiments on tumours of mice have shown lasting fluorescence of DMTC only in areas of high calcium content as indicated by the von Kossa method. However we only used a single dose of 100 mg/kg of body weight which although rather high if the LD_{50} is taken into account is less than that of most investigators (13, 21, 24, 29). Thus it may be possible to obtain fluorescence even in tissues without histological signs of calcification if higher doses are used (24).

CONCLUSIONS

A high and persisting uptake of H^3 TC and DMTC in osteosarcomas and soft tumours of mice was found to coincide exactly with the von Kossa positive parts of the tumours whereas sometimes a rather high initial accumulation but no retention was seen in von Kossa negative areas. This is in agreement with the findings of many if not all other authors that tetracycline induced fluorescence in tumour tissue as well as in other kinds of pathological tissue may be related to the calcium content of the tissue.

Our experiments further seem to confirm that the fluorescence of TC does not quite mirror its concentration. Gross fluorescence is diminished

or extinguished by high amounts of blood and by an acid pH and in addition the fluorescing structures may of course be too small to be detected macroscopically.

On the other hand a high tissue content of calcium enhances the fluorescence intensity more than can be accounted for merely by the increased concentration of the drug. This is true of normal as well as tumour tissue and it is in keeping with the results of Machado et al. (21) who investigated the distribution of H^3 TC in tumours of mice by means of tritium assay and fluorescence technique. They found much higher concentrations of H^3 TC necessary to induce fluorescence in for example liver or heart muscle than in the necrotic parts of the tumours assumed to have an increased calcium content. Further the fluorescence of aqueous solutions of tetracyclines is highly increased by the addition of calcium (or other divalent metal ions) and the fluorescence of tetracyclines in tumour tissue can be prevented by previous treatment with Na EDTA. Moreover the relatively few investigations performed with radioactive tetracycline compounds have given no unequivocal evidence of the retention of radioactivity in soft tumours (12, 14, 17, 21, 23) whereas fluorescence investigations have yielded more positive results. Eskelson et al. (14) even found other radioactive compounds to be retained in a mouse tumour to the same extent as different radio-iodinated tetracyclines.

It is concluded that persisting fluorescence of tetracyclines in tumours as in other tissues can be ascribed to the presence of calcium which is probably less important in increasing the real concentration of the drugs than in enhancing their fluorescence.

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CONCLUSIONS

A high and persisting uptake of H³TC and DMCTC in osteosarcomas and soft tumours of mice was found to coincide exactly with the von Kossa positive parts of the tumours whereas sometimes a rather high initial accumulation but no retention was seen in von Kossa negative areas. This is in agreement with the findings of many if not all other authors that tetracycline induced fluorescence in tumour tissue as well as in other kinds of pathological tissue may be related to the calcium content of the tissue.

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It is concluded that persisting fluorescence of tetracyclines in tumours as in other tissues can be ascribed to the presence of calcium which is probably less important in increasing the real concentration of the drugs than in enhancing their fluorescence.

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COMPARISON OF THE DISTRIBUTION OF DEMETHYLCHLORTETRA- CYCLINE AND RADIO CALCIUM IN WHOLE BODY SECTIONS OF TUMOUR BEARING MICE

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Abstract The fluorescence distribution of demethylchlortetracycline (DMCTC) and the autoradiographic distribution of Ca^{45} have been studied in whole body sections of mice with mammary carcinomas. High and persistent accumulation of both compounds was found only in calcified organs such as bone, teeth and tracheal cartilage. Though no gross calcification is present in the tumour as judged from the von Kossa stained sections, Ca^{45} is concentrated in the cellular tumour parts at 1 h and 5 h after injection with a decay at 12 h. Only weak radioactivity is seen in totally necrotic structureless areas. DMCTC induced fluorescence seen only in the cellular areas is neither more intense nor retained for a longer time than in many other soft tissues.

When both DMCTC and Ca^{45} are given simultaneously they interfere with each other's distribution and are for example highly accumulated in the lungs and red pulp of the spleen which suggests an intravascular complex formation. This may possibly indicate a risk of clinical side effects if tetracyclines and calcium are administered simultaneously.

Since the discovery by Rall et al (12) in 1957 of the localization of tetracycline in tumour tissue a large number of publications on the subject have appeared. Most investigators have been of the opinion that tetracycline compounds do have some kind of affinity for malignant tissue. However, very few authors have been able to find a constant and reliable uptake of the drugs in neoplasms and some have been very critical regarding the diagnostic value of the method (1, 9, 19, 20). A much discussed question has been to what components in the tumour tetracyclines are bound. Some investigators maintain that perfluorescence is seen only in macrophages, cellular debris, others that it is observed

within live tumour cells and still others that it is confined to tumour cells subject to necrosis. In favour of the latter theory is the fact that tetracyclines have been shown to be retained in a variety of non malignant necrotic tissues as well and the phenomenon has been ascribed to the increased amounts of calcium in such tissues (3, 11, 16, 17). A more thorough discussion of these matters and a review of the literature were given in an earlier paper in which also our own animal experiments were reported which favour the calcium hypothesis (5).

The subject of the present work has been to compare the distribution pictures of radio calcium and a tetracycline compound in tumour bearing mice by means of autoradiography and fluorescence technique respectively applied to whole body sections.

MATERIAL

Ca^{45} (obtained from The Radiochemical Centre, Amersham, England) was used for autoradiographic purposes and demethylchlortetracycline (DMCTC) (Ledermycin[®]) for the fluorescence studies. Nine mice bearing a mammary carcinoma (mice with mammary carcinoma SECC supplied by Dr O Sjogren, Department of Tumour Biology, Karolinska Institute, Stockholm, Sweden) induced by means of polyoma virus and transplanted through several generations were divided into three equal groups. Three mice were given Ca^{45} as a dose of 750 μ C/kg of body weight. A other four mice received 100 mg of DMCTC/kg of body weight. To the three remaining mice were administered first Ca^{45} and immediately afterwards Ca^{45} in the same dose as to the other animals. All administration was intravenous. One mouse from each group was killed at 1 h and another at 5 h and the third at 12 h after injection.

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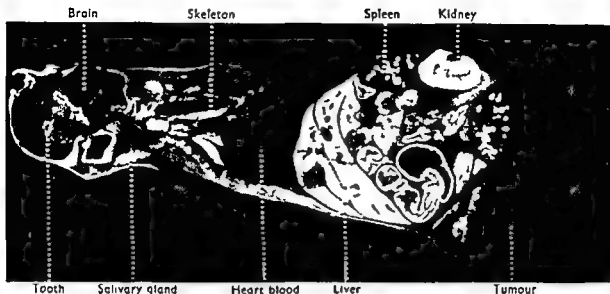


Fig 1 Fluorescence distribution of DMCTC at 1 h after injection. Note strong fluorescence in the skeleton, teeth

liver, kidney, salivary gland and white pulp and marginal zone of the spleen and weak fluorescence in the tumour.

METHODS

Killing of the mice was accomplished by immersion into a mixture of solid CO_2 and acetone (-75°C) after ether anaesthesia. At -10°C sagittal sections through the animals were cut according to the tape sectioning technique of Ullberg (18). The section thickness was $60\ \mu$ for fluorescence purposes and $0\ \mu$ for autoradiography. In the mice given both DMCTC and Ca^{45} , one $100\ \mu$ section and one immediately adjacent $0\ \mu$ section were taken at each level. After drying in the freeze room the $60\ \mu$ sections were photographed under UV light (light source Philips HPW 15 W, photographic registration obtained on Kodachrome X daylight colour film through a Kodak Wratten I filter) and the $0\ \mu$ sections were

used for autoradiography according to the method described by Ullberg (18) (exposure obtained by apposition against X-ray film (Structurix, Gevaert), time of exposure 15 days). Finally some sections from each animal were stained for calcium according to the von Kossa method.

RESULTS

Fig 1 shows the fluorographic distribution pattern of DMCTC at 1 h after injection. According to fluorescence intensity at 1 h, 5 h and 12 h after injection the organs can roughly be divided into four groups.

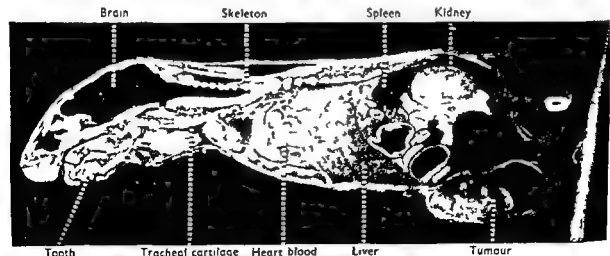


Fig 2 Autoradiographic distribution of Ca^{45} at 1 h after injection. Note high uptake in the skeleton, teeth

tracheal cartilage, intestine and cellular parts of the tumour.

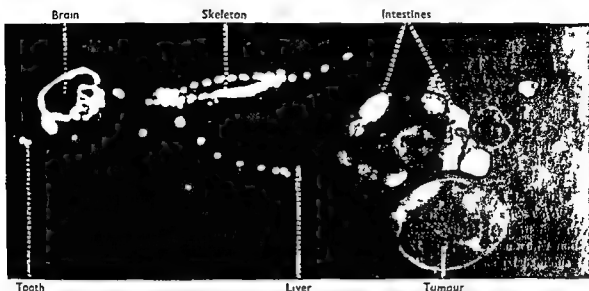


Fig 3 Autoradiographic distribution of Ca^{45} at 12 h after injection. Note high concentration in bone, teeth and intestine.

A Strong and persistent fluorescence is seen in bone, teeth and tracheal cartilage. Strong initial but somewhat decreasing fluorescence is seen in the excreting organs: liver, gallbladder and kidney.

B Fairly strong initial but rapidly decaying fluorescence is observed in the salivary glands,

lymph nodes, thymus, marginal zone and white pulp of the spleen and epididymis.

C Weak initial and rapidly decaying fluorescence is seen in the lungs, heart muscle, skeletal muscle, stomach wall, intestinal wall and contents and red pulp of the spleen. The pancreas shows persistent weak fluorescence.

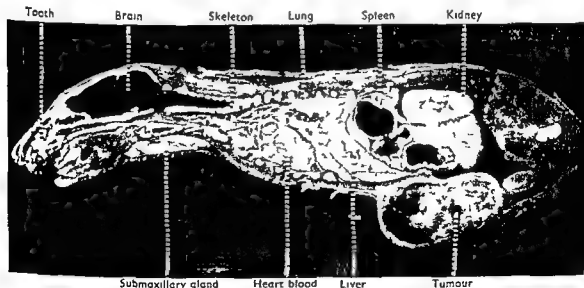


Fig 4 Autoradiogram showing the distribution of Ca^{45} at 1 h after the injection. DMCTC had been given immediately before the Ca^{45} . A higher concentration is seen in the liver, kidney, spleen, submaxillary gland and especially in the lung than is obtained with only Ca^{45} (Fig 3).

D. No fluorescence is seen in the blood CNS and teeth.

The cellular parts of the tumour have a moderate light and low continuous fluorescence whereas the non-cellular, thin tumour areas are completely non-fluorescent.

Figs 2 and 3 show the autoradiographic distribution of ^{45}Ca at 4 h and 12 h after injection in a similar way as above, the organs have been divided into four groups according to the distribution intensity on the autoradiograms from the mice killed at 4 h, 8 h and 12 h after injection.

A. High and fast uptake is seen in bone, teeth and fresh sloughs. High initial concentration with slowly decreasing level of crystal in the soft tissue and soft sloughs. Intense and well distributed puncta in soft sloughs.

B. Only high initial but rapidly decreasing concentration is seen in the blood, lungs, soft sloughs, placenta, heart muscle and kidney muscle.

C. Low initial and rapidly decreasing concentration is seen in the liver, kidney, spleen, pancreas, thymus and testis.

D. No fluorescence was visible in the CNS.

In the tumour the first part of the distribution activity at 4 h and 8 h was in the soft sloughs. In the second two parts of the distribution uptake is low but it increases slowly.

The tumour cells contain the ^{45}Ca in the soft sloughs.

When DMCTC is administered to mice the distribution of ^{45}Ca is as follows: a. In the soft sloughs (Fig. 4) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. b. In the blood (Fig. 5) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. c. In the lungs (Fig. 6) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. d. In the soft sloughs (Fig. 7) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. e. In the heart muscle (Fig. 8) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. f. In the kidney muscle (Fig. 9) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. g. In the liver (Fig. 10) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. h. In the spleen (Fig. 11) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. i. In the pancreas (Fig. 12) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. j. In the thymus (Fig. 13) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. k. In the testis (Fig. 14) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. l. In the CNS (Fig. 15) no fluorescence is visible.

The distribution of ^{45}Ca in the soft sloughs is as follows: a. In the soft sloughs (Fig. 4) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. b. In the blood (Fig. 5) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. c. In the lungs (Fig. 6) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. d. In the soft sloughs (Fig. 7) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. e. In the heart muscle (Fig. 8) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. f. In the kidney muscle (Fig. 9) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. g. In the liver (Fig. 10) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. h. In the spleen (Fig. 11) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. i. In the pancreas (Fig. 12) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. j. In the thymus (Fig. 13) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. k. In the testis (Fig. 14) the concentration is high at 4 h and 8 h and decreases slowly at 12 h. l. In the CNS (Fig. 15) no fluorescence is visible.

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DISCUSSION

The fluorographic distribution of DMCTC in normal tissues is similar to that found earlier (4). However, our experiments with tritium-labeled tetracycline (TC) have shown that the fluorescence of this compound in different organs does not quite reflect its real concentration (5) as DMCTC may well behave similarly in this respect.

Thus in the blood, lungs and heart muscle where hemoglobin quenches fluorescence the accumulation of DMCTC is probably higher than indicated by the fluorescence intensities. In the kidney the fluorescence of DMCTC because of its high resistance to acidity probably does not correspond more closely than in the case with the fluorescence of TC (6).

The autoradiographic distribution of ^{45}Ca in hard tissues is in accordance with the findings of Aperia et al. (2). However, we have not been able to detect binding of a high uptake of ^{45}Ca in the muscle considered secondary to the uptake of bone matrix in the tumour. The ^{45}Ca uptake is not a direct consequence of the high ^{45}Ca concentration in the soft sloughs but is also determined by the degree of calcification of the soft sloughs and the degree of calcification of the bone matrix.

The ^{45}Ca uptake in the soft sloughs is similar to the uptake in the bone matrix. The uptake of DMCTC in the soft sloughs is also similar to the uptake in the bone matrix.

The ^{45}Ca uptake in the blood is also similar to the uptake in the bone matrix. The uptake of DMCTC in the blood is also similar to the uptake in the bone matrix.

The ^{45}Ca uptake in the lungs is also similar to the uptake in the bone matrix. The uptake of DMCTC in the lungs is also similar to the uptake in the bone matrix.

The ^{45}Ca uptake in the soft sloughs is also similar to the uptake in the bone matrix. The uptake of DMCTC in the soft sloughs is also similar to the uptake in the bone matrix.

The ^{45}Ca uptake in the heart muscle is also similar to the uptake in the bone matrix. The uptake of DMCTC in the heart muscle is also similar to the uptake in the bone matrix.

strong fluorescence only in the von Kossa positive parts of hard and soft tumours of mice after the same dose of DMCTC as in the present work (5). It is known that the calcium content of live tumour tissue is lower than normal (6, 7, 8, 15) but rises to abnormally high values when the tissue becomes necrotic (15). Since in the tumours described in the present investigation large areas are totally necrotic and structureless and since certain parts of the cellular areas take up high amounts of calcium it may be assumed that the cells in these parts of the tumours are undergoing necrosis which has not yet reached such an advanced stage as in the structureless areas. However, fluorescence is low or very low even in these parts it is possible though that stronger fluorescence would have been obtained with higher doses than 100 mg of DMCTC/kg of body weight which is less than most other investigators have required to get lasting fluorescence (10, 12, 13, 14, 21).

When both DMCTC and Ca^{45} are given they obviously interfere with each other's distribution. Ca^{45} is now seen in organs where normally DMCTC but not Ca^{45} accumulates e.g. in the liver, kidney and lymphatic organs. The high accumulation of Ca^{45} in the lungs and red pulp of the spleen seems to indicate an intravascular formation of a complex with rather high molecular weight between Ca^{45} and DMCTC since large molecules such as colloids are known to get trapped in these organs. The lungs and red pulp of the spleen likewise exhibit stronger fluorescence of DMCTC when Ca^{45} is added which can be explained either by increased concentration of DMCTC and/or by increased degree of complex formation with calcium which is known to enhance the fluorescence of tetracyclines (5).

The risk of intravascular precipitate formation of such magnitude as to cause multiple occlusions in the capillaries of patients given calcium and tetracycline compounds simultaneously cannot be ruled out especially if both drugs are administered intravenously.

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BODY BUILD AND SERUM LIPIDS IN MALE PATIENTS HOSPITALIZED FOR PEPTIC ULCER OR MYOCARDIAL INFARCTION

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Abstract Two groups of patients have been studied. One consisted of 63 peptic ulcer patients, the other of 73 myocardial infarction patients. All patients in both groups were treated in the Medical Clinic of the Danderyd Hospital during 1965 and were followed up about one year after discharge. The infarction patients had significantly higher mean cholesterol and triglycerides than the ulcer patients (Table I). The difference occurred also between comparable age groups (Table II). The ulcer patients had a significantly higher skeletal quotient than the infarction patients, implying that the former had a longer and more slender skeletal structure. Comparison with the conscripts earlier described by Hellstrom (11) showed that in many respects the infarction patients resembled the short and heavily built conscripts, whereas the ulcer patients resembled more the tall and lightly built.

Several authors have shown that a relationship appears to exist between body build and serum lipids. Gildea et al (9) in 1936 found higher cholesterol values in the serum of 17 men with pyknic body build selected according to Kretschmer's criterion than in 24 men with leptosomic body build. In 1954 Gertler et al (8) reported that the number of mesomorphs (heavy muscles and large bones) was larger in a group of 97 men suffering from coronary heart disease than in a control group of 146 healthy men. Furthermore, in the heart disease group the mesomorphs had a higher plasma cholesterol level than the remainder of the group. In these studies the determination of body build was based either on a subjective evaluation or on a number of body dimensions which included complicated indices.

Forssman et al (6) in 1958 reported on 66 male postcoronary patients whose body build was determined on the basis of length and size of skeleton. They found that the patients with high

cholesterol values also had large length and sturdiness factors of the skeleton.

Bjurulf (2) in 1964 showed in a study of two postmortem materials that coronary atherosclerosis was more common in the short and muscular than in other men.

In 1965 Hellstrom (11) reported that conscripts 19-20 years of age with short and thick skeleton had higher serum cholesterol than conscripts with long slender skeleton. The heavily built conscripts also had a heredity of coronary heart disease which the tall and slender did not. A heredity of peptic ulcer on the other hand was rather more frequent in the tall and slender than in the short and stocky. The conscripts were grouped on the basis of body build determined according to body length and femoral condyle width, as suggested by Lindgard (16) and also on the basis of the ratio of body length to femoral condyle width; this ratio was earlier referred to by Hellstrom (10) as skeletal quotient. According to this latter grouping procedure short and thickset persons have a low skeletal quotient and long and slender a high (11).

Earlier studies thus suggest that body build has a certain relation to the serum cholesterol level. Except in the study by Hellstrom (11) however no account has been taken of the ratio of body length to femoral condyle width which should be a significant factor when deciding whether a person is heavily built or not. For this reason the skeletal quotient has been determined for all patients in the present study in which a group of peptic ulcer patients is compared with a group which had had myocardial infarction. The aim of the investigation was to study whether the find

ings in the conscripts (11) would in any way be repeated in a clinical material. The special factors of interest were therefore the body build, skeletal quotient and serum lipids of the ulcer and the infarction patients and their heredity of peptic ulcer or myocardial infarction.

MATERIAL

The material consists of two groups of patients which are compared with one another. One consists of 110 male patients hospitalized during 1965 in the Medical Clinic of Danderyd Hospital on a diagnosis of myocardial infarction. In all cases the diagnosis was based on a typical clinical course verified by ECG and elevated GOT. Of these 110 patients, 27 died during hospitalization. Of the remainder two had had diabetes mellitus and were excluded from the survey. Of the 81 patients discharged, six died before the follow-up could be made. Finally there were two patients who for different reasons could not be persuaded to come for examination. Thus, of the 81 discharged patients 73 (90%) were followed up. The other material consists of 76 men who were admitted to the Medical Clinic during 1965 on a diagnosis of ventricular or duodenal ulcer. The diagnosis was, in all cases, confirmed radiologically either as a distinct niche or as a distinct deformation of the bulb. In the original material six patients were found to have diabetes mellitus. These cases have been excluded. Since discharge from hospital five patients had died before the follow-up could be made. Furthermore two patients were lost trace of. Of the original "6 cases" there thus remain 63 (83%) all of whom were followed-up. It should be pointed out that all the patients were from the reception area of the hospital, this area which has a population of rather more than 50,000 in the northern part of the County of Stockholm, and does not cover the remainder of the county. The two series were followed up about one year after discharge and have been earlier described in detail by Hellstrom (1, 13).

METHODS

In the follow-up examination measurements were made of height, weight and blood pressure (diastolic pressure at the moment when the Korotkoff sounds rapidly diminished in loudness). The patients' ages were also recorded. Measurements were made too of the skinfold thickness on the medioclavicular line at the level of the navel and of the femoral condyle width in the right knee with the knee at an angle of 90° by the method reported earlier (15, 16, 17, 18). The ratio of height to femoral condyle width has been referred to as the skeletal quotient (10).

Samples of venous blood were withdrawn at 8 a.m. after an overnight fast. Determinations were made of cholesterol and triglyceride levels in serum by the earlier described method (3, 5) and also of the sedimentation rate and haemoglobin concentration. As the triglycerides exhibited a skewed distribution all statistical calculations have been made after logarithmic conversion of the value for the triglycerides as previously described (3, 4).

Every patient was personally questioned by the examiner whether or not he kept any form of diet, whether he avoided milk, butter and fatty food and whether he took nicotinic acid drugs. The patient was also asked whether he had previously had a gastric ulcer or myocardial infarction. Moreover the patient was asked whether he considered he was suffering particularly from mental stress at the time of onset because of his work or for other reasons and if so whether the stress was so acute that it might be considered to have been a cause of his illness. Finally all patients were asked whether either parent had been under medical care for gastric ulcer or myocardial infarction.

RESULTS

Table I presents data on the 63 ulcer and 73 infarction patients in respect of age, body dimensions, laboratory tests and blood pressures, also the differences between the two groups. The mean age of the infarction patients was found to be 11.6 years higher than that of the ulcer patients. This difference was statistically significant. As regards body build the data on height, femoral condyle width and skeletal ratio for the infarction patients were for the most part nearly comparable with those for weight lifters and wrestlers reported on by Hellstrom (10) in 1961. The ulcer patients were taller and had a smaller femoral condyle width than the infarction patients but the differences were not significant. In skeletal quotient however the ulcer patients had a significantly higher value implying that they had a longer and more slender skeleton or less heavy skeletal build than the infarction patients. The ulcer patients also had a significantly smaller skinfold thickness (Table I).

The ulcer patients had significantly lower systolic and diastolic blood pressures and cholesterol and triglyceride levels (Table I). The cholesterol and triglyceride levels for the ulcer patients were found to be largely similar to those of earlier described normal subjects of comparable age (1, 3, 4, 14, 19) whereas the infarction patients had higher values (cf 1, 3, 4). To further illustrate the difference a comparison has been made between the cholesterol and triglyceride levels of the ulcer and the infarction patients in comparable age groups. The result is shown in Table II. The ulcer patients even in comparable age groups were found throughout to have lower cholesterol and triglyceride levels than the infarction patients. The material has also been grouped according to high and low cholesterol and tri-

Table I Differences between patients hospitalized for peptic ulcer and myocardial infarction

	Patients with peptic ulcer				Patients with myocardial infarction				Diff	P
	n	\bar{X}	sD	e	n	\bar{X}	sD	e		
Age years	63	49.0	13.3	1.7	73	60.6	9.6	1.1	-11.6	
Height cm	63	175.1	7.7	1.0	73	173.3	6.2	0.7	+1.8	>0.1
Femoral condyle width cm	63	10.1	0.5	0.1	73	10.14	0.41	0.05	-0.04	>0.2
Skeletal quotient	63	17.4	0.8	0.1	73	17.0	0.7	0.1	+0.4	
Body weight kg	63	74.0	12.0	1.5	73	76.6	9.9	1.2	-2.6	>0.1
Skinfold thickness mm	63	17.6	8.1	1.0	73	23.8	7.0	0.8	-6.2	
Systolic B.P. mm Hg	63	139.1	23.1	2.9	73	153.2	25.3	3.0	-14.1	
Diastolic B.P. mm Hg	63	89.2	12.1	1.5	73	97.1	13.8	1.6	-7.9	
Cholesterol mg/100 ml	63	249.0	50.0	7.0	73	285.8	53.6	6.3	-36.8	
Log triglycerides	63	1.94	0.30	0.03	73	2.18	0.24	0.03	-0.24	
ESR mm/h	63	8.8	10.2	1.3	73	14.8	18.6	2.2	-6.0	
Hb conc g/100 ml	63	14.7	1.2	0.1	73	14.6	1.2	0.1	+0.1	>0.2

Table II Mean cholesterol and triglycerides of patients hospitalized for peptic ulcer and myocardial infarction by age groups

Age (y)	Patients with peptic ulcer			Patients with myocardial infarction		
	n	Mean cholesterol (mg/100 ml)	Mean log triglycerides	n	Mean cholesterol (mg/100 ml)	Mean log triglycerides
0-35	9	231	1.93	—	—	—
36-45	18	258	1.96	5	292	2.14
46-55	16	245	1.96	20	297	2.24
56-65	14	248	1.91	23	293	2.14
66-83	6	252	1.89	25	268	2.18

glyceride levels as has been done in earlier investigations (7-14). Here it was found (Table III) that 48 (76.1%) of the ulcer patients had normal cholesterol and normal triglycerides whereas the corresponding figures for the infarction patients were 30 (41.0%). The mean value for the triglycerides measured in mg/100 ml was for the ulcer patients 98.6 (sD 43.5) and for the infarction patients 177.4 (sD 109.8). The high sD values indicate a skew distribution of the triglycerides. Consequently all statistical calculations were made after logarithmic conversion.

Table III High and low cholesterol and/or triglyceride levels in patients hospitalized for peptic ulcer and myocardial infarction

	Patients with peptic ulcer				Patients with myocardial infarction			
	n	Age (y)	Mean cholesterol (mg/100 ml)	Mean log triglycerides	n	Age (y)	Mean cholesterol (mg/100 ml)	Mean log triglycerides
Cholesterol <300	48	47	234	1.87	30	62.2	250	1.99
Triglycerides <150								
Cholesterol >300	6	43	337	1.94	10	61.7	323	2.01
Triglycerides <150								
Cholesterol <300	7	54	260	2.31	15	60.6	258	2.38
Triglycerides >150								
Cholesterol >300	2	45	350	2.30	18	57.4	347	2.41
Triglycerides >150								

Table IV *Mental stress earlier peptic ulcer and earlier myocardial infarction in male patients hospitalized for peptic ulcer and myocardial infarction*

	Mental stress	Earlier peptic ulcer	Earlier myocardial infarction
Patients with peptic ulcer n=63	41 (65.0%)	41 (65.0%)	0
Patients with myocardial infarction n=73	42 (57.5%)	11 (15.1%)	11 (15.1%)

sion of the value for the triglycerides as earlier reported (3, 4)

Of the ulcer patients nine (14.1%) stated that they kept a moderate ulcer diet and three that they particularly avoided fats. These patients did not materially differ from the others in blood fats, body weight or skinfold thickness (12). Similar results were obtained for the infarction patients. The 49 myocardial infarction patients who stated that they particularly avoided fats had as high blood fat values and skinfold thickness as the other 24 patients in the same group who kept no diet (13).

No significant correlation was found for either the ulcer or the infarction patients between cholesterol or triglycerides and age, weight, height, femoral condyle width, skeletal quotient or skin fold thickness.

Mental stress (Table IV) was considered to have been present to such a degree that it contributed actively in bringing on the illness in 65.0% of the ulcer and in 57.5% of the infarction patients. No less than 65.0% of the ulcer patients had earlier had a peptic ulcer but none a myocardial infarction. Among the infarction patients (Table IV) 15.1% had had an earlier peptic ulcer and an equal number an earlier infarction.

Table V *Patients reporting a history of myocardial infarction or gastric ulcer in a parent*

	Peptic ulcer	Myocardial infarction
Peptic ulcer patients n=63	18 (28.5%)	4 (6.3%)
Myocardial infarction patients n=73	6 (8.2%)	11 (15.1%)

One parent of 28.5% of the ulcer patients had had a peptic ulcer and one parent of 6.3% an infarction. The corresponding figures for the infarction patients were 8.2 and 15.1% (Table V).

DISCUSSION

The 73 infarction patients had so short and heavy a skeletal structure that they were virtually comparable in this respect to weight lifters and wrestlers earlier reported on by Hellstrom (10). The 63 ulcer patients moreover had a significantly higher skeletal quotient than the infarction patients implying that they had a longer and narrower skeleton. This difference in skeletal structure must be regarded as due to differences in the hereditary factors which determine skeletal build.

The infarction patients had significantly higher mean cholesterol and triglyceride levels than the ulcer patients (Table I). Differences appeared also between comparable age groups (Table II). Thus some 34% of the ulcer and 59% of the infarction patients had elevated blood fats (Table III).

No less than 65.0% of the ulcer and 57.5% of the infarction patients considered they had been in a state of mental stress which had actively contributed to their illness (Table IV). Thus mental stress seems to play an important role in the development of peptic ulcer and myocardial infarction. Of the ulcer patients 65.0% had had a previous peptic ulcer but none a myocardial infarction. Furthermore one parent of 28.5% of the ulcer patients had had a peptic ulcer and one parent of 6.3% a myocardial infarction. Only 15.1% of the myocardial infarction patients had had a previous peptic ulcer and the same number a previous myocardial infarction. 8.2% of the parents of these patients had had peptic ulcer and 15.1% myocardial infarction (Table V). These figures are perhaps evidence of an inherited tendency to react to mental stress in a particular way.

The findings for the 63 ulcer and 73 infarction patients closely resemble those for the conscripts reported on by Hellstrom earlier (11). The infarction patients had a shorter and sturdier skeletal build and higher serum cholesterol level than the ulcer patients and a greater number of infarctions among the parents whereas a larger number of the parents of the taller and slimmer ulcer patients had suffered peptic ulcer.

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THE NORMAL METABOLISM OF γ G GLOBULIN

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Abstract A group of normal subjects has been studied after careful clinical examination. Different methods of determining the intravascular γ G globulin concentration and of calculating the catabolism were used. The difference in the values as well as the composition of the material may well explain the divergent results published earlier. A standardization of these methods is therefore proposed. By using whole body counting as well we obtained further information which showed that the plasma and the whole body curves are parallel but not the plasma and the retained dose curves. This must signify that if incomplete urine collection has been excluded ^{125}I eliminated by routes other than the urine. About 17% of the total daily amount of eliminated radioactivity can be estimated to be lost by routes other than the urine which in turn means that the catabolic value will be too low when calculated by the U/P method. Possible routes of the ^{125}I -elimination would be perspiration and via the saliva. Measurement of the extravascular pool by whole body counting showed that in healthy active subjects the extravascular compartment is equal in size to the intravascular.

During the last five years the metabolism of γ G globulin in control subjects has been studied by some authors (1, 2, 4, 5, 12). The results do not agree completely, however. Moreover some important questions are still unsolved. So far it has not been established where the γ G metabolism takes place or whether the activity excreted in the urine reflects the whole excretion nor is the true relationship between the extravascular and intravascular pools known.

We present here a comparative study of the metabolism of γ G globulin based on data obtained by whole body counting as well as by serum and urine analyses. Such a study would provide further information that may explain the differences between the published metabolic values of control subjects and allow a comparison between values obtained by different methods of calculation and of measuring the serum protein

MATERIAL AND METHODS

The study comprised seven healthy volunteers (three men and four women) whose ages ranged between 17 and 44 years. All had undergone careful medical examination including several laboratory tests. None of them had any acute or chronic infections or other pathological processes which might influence the γ G globulin situation and conventional blood and urine analyses were normal in all cases. The relative electrophoretic serum protein distribution as well as the haematocrite values were normal throughout. The results of these analyses were checked several times during the metabolic study.

The subjects were investigated in the outpatient department and all carried on their ordinary work in the hospital during the period of study. Three of the women menstruated once and one twice during the period of urine collection.

Protein methods

γ G globulin was prepared from pooled normal sera by chromatography on DEAE-Sephadex followed by gel filtration on Sephadex G 00. Phosphate buffer 0.05 M pH 8.0 was used.

Labelling with ^{125}I was done by McFarlane's method (6). The labelled protein preparation was tested for sterility and pyrogens.

Quantitative determination of γ G globulin in serum was done by (1) Determination of the gammaglobulin fraction by (a) paper electrophoresis (b) free boundary electrophoresis by the Tiselius method and (2) the single radial diffusion method described by Mancini et al. (8). Total serum protein values were determined by a biuret method. Comparisons were made on means of repeated samples.

Performance of the isotope study

About 20 μCi ^{125}I labelled γ G globulin (equalling at the most a few m μ of γ G globulin) was injected intravenously. Blood samples were taken 10 minutes after the injection and then daily for two weeks thereafter every second to third day. The plasma volume was calculated by the isotope-dilution technique. The subjects were carefully instructed to save all the urine for each 24-hour period. From these amounts samples were taken for measurements of radioactivity. Usually the urinary activity could not be measured with satisfactory precision after 45 days.

Whole body counting was done one and three hours after the injection of the isotope and then simultaneously with the blood samples

In all cases sodium iodide solution (ten drops of 10% NaI daily) was given three days before and throughout the period of investigation which covered 50 days

Methods for calculation of metabolic data

Mathematical analysis of the plasma activity curve was done by Matthews method (9). In addition the 24-hour urinary excretion of activity (U) was divided by the mean activity in plasma during 24 hours (P). The resulting ratio U/P is a measure of the catabolism and is constant throughout the period of study if the breakdown occurs in the intravascular pool or in a compartment with rapid exchange with the intravascular space.

The excretion of activity was obtained also by the differences between successive whole-body counts. The ratio of the difference between two successive whole body counts to the plasma activity can be used to obtain a measure of the catabolism.

The calculations were made with a digital computer with two ALGOL programs PLASMA I and PLASMA II (10). All U/P and whole body calculations were performed on measurements between days 5 and 26.

Methods for determination of whole body activity

The patient lies supine on a stretcher in a steel walled room. One scintillation detector with a sodium iodide crystal moves above and another below the stretcher. All pulses over 0.1 Mev were counted in order to obtain sensitivity. The patient's background activity was measured before injection of isotope. The efficiency of measurement of ^{131}I was about 1%. Further details about the whole body counter are published elsewhere (11).

Measurement of radioactivity

Four ml of plasma or urine were measured in a well scintillation counter. Only the activity in the photopeak at 0.36 Mev for ^{131}I was recorded. The activity in menstrual fluid was analysed by measuring the sanitary towels

in a special liquid scintillation counter (Packard Armac extra large).

Definitions

Catabolism The normal or pathological process by which the molecules of a substance are converted to other molecules or ions.

Fractional catabolic rate Catabolism per unit of time expressed as a percentage of the intravascular pool. If the size of this pool is known the catabolism can be expressed in g per day.

Retained dose The body's total activity calculated by subtracting the total urinary and faecal excretion of activity from the given dose.

Whole body activity The body's total activity measured with a whole body counter.

Pool The area or the form in which a substance occurs. A pool may be a physiologically or chemically defined form. The feasibility to define a pool is dependent on its rate of exchange with other pools in relation to sampling intervals and the time of investigation.

RESULTS

Table I shows the total serum protein value and the γ globulin concentration in serum determined by paper electrophoresis, free boundary electrophoresis and immunologic analysis. The plasma volume was also recorded as was the intravascular content of γ globulin which was calculated by the three methods. The γ globulin values obtained by paper electrophoresis were highest while free electrophoresis and immunologic analysis of γ globulin gave virtually identical mean values. The γ globulin values obtained by paper electrophoresis are 8–10% higher than the other values. The difference between this

Table I Gamma G globulin concentration and intravascular (^{131}I) gamma-G globulin content according to different methods in seven normal subjects

Case no	Total protein (g/100 ml)	Gamma G globulin (g/100 ml)				IV gamma-G globulin (g)		
		Electrophoresis			Plasma vol (l)	Electrophoresis		
		Paper	Free boundary	Mancini method		Paper	Free boundary	Mancini method
1	8.2	1.29	0.98	1.24	3.10	40.0	30.4	38.4
2	7.9	1.08	0.93	0.90	2.82	30.5	26.2	25.3
3	7.7	1.04	0.92	0.98	3.01	31.3	27.7	29.3
4	7.9	0.91	1.23	0.83	3.00	27.3	36.9	24.8
5	7.5	1.07	1.15	1.07	3.67	38.7	41.6	36.8
6	7.9	1.18	1.08	0.98	1.98	23.4	21.4	19.4
7	8.1	1.05	0.75	0.98	2.67	28.0	20.0	26.1
Mean	7.9	1.09	1.01	0.99	2.89	31.3	29.2	28.6
SD	0.2	0.12	0.16	0.13	0.50	6.1	7.9	6.8

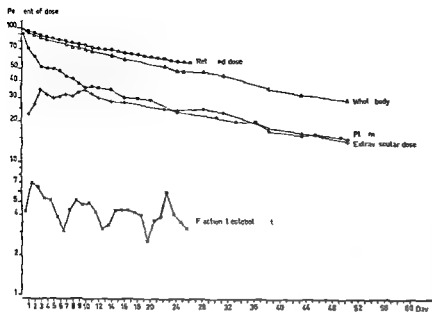


Fig 1 The catabolism of γ G globulin in a normal subject (The fractional catabolic rate is expressed in % of intravascular pool per 24 h)

method and the immunologic method in this respect is significant ($p < 0.01$). The difference between the values obtained by the different quantitation methods are of course reflected in the figures obtained from calculation of the intravascular γ G globulin concentration.

Fig 1 shows the results from a study of one of the subjects. In this as in the rest of the investigated cases there is a constant catabolism which indicates that non denatured protein preparations were used. Neither in this nor in the other cases were the curves for retained dose and plasma activity parallel. The whole body analyses

showed in all cases a minor but unmistakable discrepancy between the whole body and the retained dose curves. The plasma curve, extravascular curve and the whole body curve on the other hand run parallel throughout the latter part of the analyses.

As will be seen from Table II the catabolism was calculated by three different methods. The fractional catabolic rate obtained by mathematical analysis was virtually identical to that calculated from whole body data. The U/P analysis, which is based on urine measurements on the other hand gave lower values with significant deviation

Table II Catabolism and extravascular/intravascular distribution (EV/IV) of gamma-G globulin according to different methods in seven normal subjects

Case no	Catabolism									EV/IV	
	U/P (/day)	Matthews	Whole body	U/P (g/24 h)	Matthews	Whole body	U/P (mg/24 h/kg)	Matthews	Whole body	Whole body	Matthews
1	4.8	5.7	5.7	1.84	2.19	2.18	27	32	32	0.64	0.83
2	4.6	5.9	5.4	1.17	1.49	1.37	17	21	20	1.07	1.13
3	3.9	5.4	5.0	1.13	1.58	1.46	20	29	26	1.02	0.96
4	4.2	4.7	5.0	1.03	1.17	1.24	18	14	15	0.97	0.87
5	4.8	6.5	6.4	1.75	2.39	2.37	18	25	24	1.07	0.83
6	3.7	4.8	4.8	0.72	0.93	0.93	15	19	19	1.05	0.99
7	6.6	7.4	7.6	1.73	1.93	1.99	22	25	26	1.07	1.17
Mean	4.65	5.77	5.70	1.34	1.67	1.65	19	23	23	0.98	0.97
SD	0.97	0.95	1.01	0.43	0.53	0.53	5.0	6.1	5.7	0.15	0.14

Table III Per cent of injected dose distributed intra (IV) and extravascularly (EV) excreted and retained in the body according to two calculation methods in seven normal subjects

	IV	Retained dose	Total urinary excretion	EV from retained dose	Whole body activity	EV from whole body activity	Difference between retained dose and whole body activity
Day 5							
Mean	46.0	79.7	20.3	33.7	76.6	30.6	3.1
s.d.	3.89	6.21	3.84	2.61	4.46	2.96	0.89
Day 25							
Mean	21.2	52.1	47.9	30.8	42.2	21.0	9.8
s.d.	3.06	6.41	6.30	4.15	6.46	4.20	1.47
Day III							
Mean	15.2	—	—	—	31.1	15.9	—
s.d.	3.07	—	—	—	5.99	3.72	—

in comparison with both the whole body method and with Matthews method ($p < 0.001$). This of course influences the metabolic figures expressed both as μ per 24 hours and as mg per 24 hours per kg.

Table III shows the intravascular and extravascular distribution calculated from retained dose and whole body activity 5, 25 and 38 days after the start of the analyses. The table does not record any retained-dose data at 38 days as the elimination via the urine could not be determined with sufficient accuracy at this time.

After five days there was still much more γ G globulin intravascularly than extravascularly. A difference of 3.1% of the given dose was noted between the activity remaining in the body calculated as retained dose and estimated as whole body activity. After 25 days there was as much

γ G globulin intravascularly as extravascularly if the calculation is based on whole body data or mathematical analysis. The extravascular distribution and activity remaining in the body calculated as retained dose showed values that were 9.8% higher than those determined by whole body analysis which can be interpreted as an indication that this activity left the body by routes other than the urine.

DISCUSSION

The results of five earlier studies with non denatured γ G globulin and the results of the investigation presented here are compiled in Table IV.

It will be seen from Table IV that divergent results have been obtained for catabolism expressed in per cent per day as well as in g per

Table IV Catabolism of gamma-G globulin according to different authors

Authors	No of cases	Type of subjects	Catabolism					(g/24 h)	(mg/24 h/kg body weight)
			(/24 h)						
			Methods of calculation ^a						
			A	B	C	D	E		
Cohen & Freeman (5)	5	Normals	5.2	5.8	5.1		3.0	2.1 ^b 2.4 (E)	
Birke et al (4)	10	Controls			4.8			1.7 (C)	25
Solomon et al (12)	14	Controls					3.0	2.3 (E)	36
Ahlinder et al (1)	15	Controls			4.8			1.8 (C)	25
Andersen (3)	21	Convalescents			6.9			2.5 (C)	36
Present invest	7	Normals		5.8	4.7	5.7		1.7 (B) 1.3 (C) 1.7 (D)	23 19 23

^a A = equilibrium time B = Matthews C = U/P D = Whole body counting

E = From $T_{1/2}$ of plasma activity curve and total body gamma G globulin pool

^b Calculated from mean of methods A, B and C

day and mg per kg per day. These discrepancies might have several causes which are worth discussing. Catabolism expressed in g per 24 hours or in mg per kg per 24 hours depends partly on the method used for determining the γ G globulin concentration in serum. Therefore we determined the γ G globulin concentration by three different methods. Paper electrophoresis yields values that are 8–10% higher than those obtained by the other two methods. The difference in this respect between paper electrophoresis and immunologic analysis is significant, whereas free boundary electrophoresis gives numerically lower values without statistical significance in comparison with the values of paper electrophoresis. The difference between paper electrophoresis and the other two methods may be expected to be higher and assume still greater importance in studies of patients with dys γ globulinaemia, especially those with hypo- or a γ globulinaemia.

The free boundary electrophoresis and the immunologic methods gave virtually the same mean values in this study. In our previous analyses we used free electrophoresis but in view of the similar results obtained by the immunologic method and as this analysis is fairly simple to perform and does not require expensive equipment, we employed it in the present study and shall continue to do so.

The second factor that influences the results is the choice of method for calculation of the catabolism expressed in per cent per day. The urine/plasma (U/P) analysis gives a value of 4.7% which is in agreement with that previously reported by us in control subjects of varying ages but slightly lower than Cohen and Freeman's data (5) and distinctly lower than those of Andersén's control cases (3). All three authors use the same method of calculation. Solomon et al.—and also Cohen and Freeman—on the other hand calculate the catabolism from the slope of the plasma disappearance curve and the total gamma globulin pool. Their result 3% per day is slightly higher than the average of the other methods if an equal distribution of the gamma globulin between intra- and extravascular pools is assumed. The mathematical and the whole body analysis both give catabolic rates of about 5.7% per day which is about 21% higher than the value obtained by the U/P method. This difference is significant. The causes and the importance

of this difference will be discussed later on but it is worth mentioning that whole body counting and Mathews' method give similar values. Thus the method of calculating the catabolism and of determining the serum γ G globulin concentration may explain some of the varying results reported for control cases.

The lack of parallelism between the plasma curves and the retained-dose curves which was noted in these and in previously reported control cases should be discussed as its interpretation may be thought to influence the results.

The whole body count was made on the whole gamma spectrum and not on the photopeak alone. Thus redistribution errors were minimized. Control measurements have indicated that these errors were well below one per cent (11). Therefore it does not seem probable that the technical performance of whole body measurement influenced the values obtained.

Accumulation of ^{131}I in the body as iodide is improbable. The thyroid uptake was blocked by inert iodine in this and in other investigations. Studies of larger control series have shown that under this condition <1% is taken up by the thyroid. Cohen and Freeman studied the elimination of ^{131}I and showed that it is neither taken up by nor fixed in any other tissue of the body (5). This was confirmed by whole body counting in one of our cases given ^{131}I (2). Our studies with ^{131}I tyrosine (moniodotyrosine) show some delay of elimination but no accumulation (2). As there is thus no accumulation of ^{131}I metabolites in control cases there may be three alternative explanations of the lack of parallelism between the retained-dose and the plasma curves: 1. incomplete collection of urine; 2. losses of ^{131}I by routes other than the urine; or 3. a globulin pool that is slowly exchangeable in relation to the period of investigation.

It is evident from the data that the difference between the retained-dose and the whole body curves increases each day by a certain amount and reaches a maximum of 9.8% of the given dose after 25 days. This means that about 17% of the total daily loss of activity occurs by routes other than the urine. The whole body and the plasma curves are parallel which signifies that when urinary collection is incomplete in these—as in the previously reported cases—exactly the same proportion of the 24 hour urine is lost each day.

THE TUBULAR REABSORPTION OF CALCIUM IN PRIMARY HYPERPARATHYROIDISM AND IN NON PARATHYROID HYPERCALCEMIA¹

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Abstract The tubular reabsorption of calcium, expressed in percentage of the filtered load of calcium (TRCa%) and the phosphate excretion index were examined in 31 hypercalcemic patients to evaluate their relative value in the differential diagnosis of hypercalcemia. The phosphate excretion index proved to be of no value.

High TRCa% values were found in primary hyperparathyroidism (15 pat.) in hyperparathyroidism and sarcoidosis (1 pat.) and in hypercalcemia attributed to metabolic alkalosis (7 pat.). The elevated TRCa% values in the last named condition are probably explained by a specific effect of the metabolic disturbance on the renal tubule. It is inferred that an increased tubular reabsorption of calcium may contribute to the development and maintenance of the hypercalcemia in the milk alkali syndrome.

Low TRCa% values were found in poststrumectomy hypoparathyroidism overdosed with vitamin D (3 pat.) ordinary hypercalcemic sarcoidosis (5 pat.) idiopathic intestinal hyperabsorption hypercalcemia (1 pat.) Hodgkin's paragranuloma (1 pat.) myelomatosis (1 pat.) and surprisingly in hyperparathyroidism associated with sarcoidosis (1 pat.) and with idiopathic hypercalcemia (1 pat.).

It is concluded that TRCa%—with only a few exceptions—reflects the functional state of the parathyroid glands in hypercalcemia being elevated in the presence of hypersecretion of parathyroid hormone and depressed in non parathyroid hypercalcemia in which functional hypoparathyroidism is assumed to be present.

In primary hyperparathyroidism the 24-hour renal excretion of calcium is of minor diagnostic importance since normal excretion as well as raised excretion are frequently found (38-56). The urinary calcium excretion depends on glomerular filtration and tubular reabsorption and is thus influenced by the concentration of ultrafiltrable

calcium in plasma. The glomerular filtration rate is 3 factors affecting the capacity of tubular reabsorption. The ultrafiltrable fraction (UF_{Ca}) of the total serum calcium comprises a large free or ionized part (Ca^{++}) and a small complex bound one of which the latter is less completely reabsorbed (33-34-59). However the complex bound fraction undergoes unpredictable changes in the tubules and therefore it is only possible to determine the net reabsorption of the total ultrafiltrable fraction. The reabsorption comprises from 94 to over 99% of the filtered calcium (14-36-38-40-48) it is active (14-42) and takes place along the entire nephron (34-42). About 70% of the reabsorption takes place in the proximal tubules 20-25% in Henle's loop and 10% in the distal tubules and the collecting ducts (42). An increase in the filtered load of calcium raises the absolute amounts of calcium reabsorbed (14-36-46) but the reabsorption in percentage of the filtered load decreases (38-46). No T_m value for the reabsorption of calcium can be demonstrated (14-46). The calcium reabsorption in the proximal tubules is enhanced by the proximal reabsorption of sodium and is therefore influenced by variations in this parameter (18-39-59).

The parathyroid hormone increases the tubular reabsorption of calcium as is demonstrated in rats (53-54) in dogs (60) and in man (4-38). The parathyroid hormone seems to increase the reabsorption in the distal tubules only (18-60). The effect of thyrocalcitonin on the tubular reabsorption of calcium is still unknown.

Against the background of the well-established feed back interplay between Ca^{++} and the para-

¹ Presented in part at the IVth International Sarcoidosis Conference September 1966 Paris and at the Vth Acta Endocrinologica Congress August 1967 Helsinki.

Table 1 Summary of the dietary intake of calcium and phosphorus and those serum and urine parameters essential for the calculation and interpretation of the tubular reabsorption of calcium (TRCa%) and the phosphate excretion index (PEI) (Thirty-one patients with hypercalcemia and four normal subjects)

Pat no	Initials	Sex	Age	Diet		Serum		Urine										
				Type	Ca cont (mg/24 h)	Ca cont	P cont	Total Ca (mg/100 ml)	Ca	UFCa	Stand bic (mEq/l)	pH capill blood	CCr (ml/min)	Ca (mg/24 h)	TRCa (%)	24 h P E I -0.08- +0.09	Na (mEq/24 h)	
Primary hyperparathyroidism																		
1	BSH	♀	24	C	—	—	3.0	11.00	7.25	8.50	22.4	7.42	83.0	153	98.5	0.00	—	
2	JAN	♂	24	B	941	1180	2.8	11.17	7.55	8.50	24.0	7.41	89.9	160	98.6	+0.20	45	
3	IT	♂	53	C	—	—	3.7	12.80	8.60	9.80	4.5	7.41	81.7	408	98.2	+0.31	—	
4	JM	♂	29	B	864	1200	2.7	11.55	7.95	8.75	23.2	7.41	152.4	594	97.3	+0.10	105	
5	GMP	♀	57	C	—	—	3.5	14.70	9.90	11.45	35.7	7.45	128	178	96.0	+0.48	—	
6	AHJ	♀	48	B	917	1055	2.6	17.15	11.85	13.03	22.4	7.47	42.5	363	95.5	+0.30	45	
7	NKC	♂	62	B	724	1121	2.9	11.00	7.45	8.35	23.8	7.39	82.8	162	98.4	+0.09	60	
8	AHO	♂	52	B	810	—	2.2	14.25	10.15	11.00	24.0	7.43	82.0	472	96.4	+0.33	54	
9	EXJ	♀	56	C	—	—	2.4	11.60	7.85	8.80	22.5	7.42	70.7	105	98.8	+0.20	—	
10	KJ	♂	55	B	705	1173	3.4	11.40	7.35	8.60	23.0	7.40	105.2	366	97.1	+0.09	—	
11	MH	♂	48	C	—	—	2.9	11.15	6.98	7.88	14.8	7.39	150.8	488	97.2	+0.10	—	
12	EE	♀	58	C	—	—	2.8	10.60	6.70	7.55	4.0	7.47	85.3	190	98.0	+0.07	—	
13	CWJ	♂	51	A	793	1070	3.1	15.02	9.93	11.36	23.0	7.45	18.4	149	97.6	+0.37	57	
14	EJP	♂	41	A	804	1051	2.0	14.80	10.75	11.93	22.6	7.35	78.6	337	97.5	+0.36	72	
15	VPS	♂	45	C	—	—	2.9	10.15	6.75	7.65	23.9	7.40	108.4	565	95.3	+0.12	—	
16	HH	♂	72	C	—	—	3.3	9.85	6.68	7.52	23.3	7.18	50.8	123	97.7	+0.14	—	
Hyperparathyroidism associated with sarcoidosis																		
17	MEJ	♀	67	B	901	1156	3.0	12.00	8.35	9.30	22.5	7.39	61.9	176	97.9	+0.70	63	
18	BES	♀	23	C	—	—	2.1	11.80	8.05	9.15	23.9	7.46	45.1	386	93.5	+0.42	11	
—	—	—	—	A	870	1096	2.7	12.64	8.54	9.95	27.1	7.48	49.3	356	94.9	+0.38	72	
Ordinary hypercalcemic syndromes																		
19	L/K	♀	33	C	—	—	3.3	13.00	8.25	10.05	23.6	7.39	33.2	779	83.7	+0.33	104	
—	—	—	—	A	839	1109	3.5	11.19	7.61	8.90	23.2	7.39	13.7	594	86.1	+0.45	77	
20	ABH	♂	69	C	—	—	3.7	14.50	—	11.42 ^a	—	—	29.7	781	84.0	+0.38	—	
21	KMC	♂	33	C	Anorexia Vomiting	—	4.4	13.63	9.55	10.88	26.4	7.43	32.9	517	90.1	+0.27	61	
22	LKK	♂	25	C	—	—	2.6	10.90	7.10	8.90	14.1	7.43	109.3	582	96.0	—	—	
—	—	—	—	A	1117	1179	3.1	11.78	8.04	9.49	24.5	7.42	84.5	539	95.3	+0.13	—	
23	TFS	♂	6	C	—	—	4.2	11.25	7.80	8.45	25.1	7.43	48.9	422 ^b	90.0	+0.21	—	
—	—	—	—	B	705	1173	4.5	10.63	7.25	8.18	27.7	7.45	72.8	544	95.8	+0.06	33	
—	—	—	—	A	740	1177	4.5	10.97	7.23	8.47	25.1	7.42	68.7	376	95.5	+0.04	31	

3	PCO ₂	5	821	1114	42	1156	760	885	789	747	736	361	880	+0.37
6	ED	1	778	1156	30	1156	760	885	789	747	736	361	880	+0.37
6	NaH ₂ PO ₄	46	—	—	30	1160	805	915	—	—	—	436	880	+0.42
27	Idiopathic intestinal hyperabsorption hypercalcemia	—	—	—	32	1080	708	797	250	741	1176	732	944	+0.08
27	HOCN	44	—	—	36	1060	727	801	39	718	1147	616	953	+0.01
28	Hodgkin's paraneoplasia	—	—	—	—	—	—	—	—	—	—	—	—	—
28	DSH	78	—	—	36	990	660	770	256	747	753	371	953	+0.08
28	DSH	—	—	—	39	1340	791	943	255	741	233	365	885	+0.36
29	Myelomatosis	—	—	—	—	—	—	—	—	—	—	—	—	—
30	VO	67	—	—	40	1190	890	1010	255	743	528	318	953	+0.11
30	SHC	39	1177	1314	42	1033	690	843	321	746	766	56	994	-0.03
31	ECMH	44	1240	1357	28	1140	735	885	783	749	746	73	992	+0.16
32	Normal subjects	—	—	—	—	—	—	—	—	—	—	—	—	—
32	APF	70	749	1107	42	1000	648	745	740	741	1074	187	984	-0.06
33	JG	23	807	1163	40	985	685	730	245	741	1139	200	984	-0.03
34	SM	71	807	1163	37	990	638	740	743	740	1204	214	983	-0.01
35	LH	4	807	1163	39	960	—	718	238	740	1156	243	980	+0.02

UF/Ca was calculated from the total calcium concentration by use of the mean total calcium/UF/Ca ratio in ordinary hypercalcemic sarcoidosis

thyroid hormone secretion (51) it may be surmised that hypercalcemic subjects have either increased or decreased secretion of parathyroid hormone. The secretion will be raised in primary hyperparathyroidism (5) and in malignant tumors producing parathyroid hormone-like substances (55) while a decreased secretion must be expected in hypercalcemia of non parathyroid origin. As the parathyroid hormone increases the tubular reabsorption of calcium it may thus be anticipated that raised tubular reabsorption is found in hypercalcemia owing to primary hyperparathyroidism and reduced tubular reabsorption in hypercalcemia of non parathyroid origin. Preliminary observations have been in accordance with this hypothesis (57). The present publication represents a further testing of the hypothesis.

MATERIAL

The study comprises thirty-one hypercalcemic patients and four normal subjects (Table 1). Hypercalcemia is here defined by the presence of an increased concentration of Ca^{++} . There were the following causes for hypercalcemia: primary hyperparathyroidism (16 pat), hyperparathyroidism associated with sarcoidosis (2 pat) or dietary hypercalcemic sarcoidosis (5 pat), post-tracheotomy hyperparathyroidism overdosed by vitamin D (3 pat), idiopathic intestinal hyperabsorption hypercalcemia (1 pat), hypercalcemia due to Hodgkin's paraneoplasia (1 pat), myelomatosis (1 pat), and hypercalcemia associated with metabolic alkalosis and hypokalemia (1 pat). The latter condition was due to vomiting in anorexia nervosa (case no 30) and to overtreatment with sodium bicarbonate in renal tubular acidosis (case no 31). The diagnosis of primary hyperparathyroidism was verified histologically by parathyroidectomy (cases no 1-13, 17-18) and by a biopsy in generalized osteitis fibrosa (case no 14). Finally in cases no 15-16 a histologically negative neck exploration was followed by a lasting normalization of Ca^{++} . The diagnosis of sarcoidosis, Hodgkin's paraneoplasia and myelomatosis was confirmed histologically. Further information, concerning the patients with idiopathic intestinal hyperabsorption hypercalcemia, with the combination of hyperparathyroidism and sarcoidosis and with some of the other conditions, will be found in earlier publications (4, 5, 31, 33, 57, 58). The initials of the patients can be used for identification. Separate papers will appear on the serum calcium fractions in hypercalcemia and on idiopathic intestinal hyperabsorption hypercalcemia, ordinary hypercalcemic sarcoidosis, Hodgkin's paraneoplasia, hyperparathyroidism associated with sarcoidosis and metabolic alkalosis.

METHODS

Attempts were made to perform the examinations under normal intake of calcium, phosphorus, sodium and protein. In the majority of the patients examined the

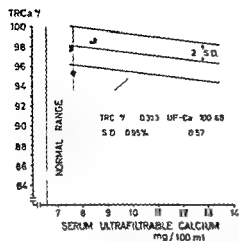


Fig 4 The tubular reabsorption of calcium (TRCa %) related to the concentration of ultrafiltrable calcium in serum. Sixteen patients with primary hyperparathyroidism (●) and two patients with sarcoidosis and hyperparathyroidism (x). The patient with primary hyperparathyroidism and idiopathic hypercalcaemia is indicated by a circle (see text).

primary hyperparathyroidism. Some of the patients were examined at different levels of UFCa or on several admissions, which is indicated by a line between the TRCa % values in question. Generally the TRCa % tended to be lower at the higher concentrations of UFCa, but great individual variations are seen.

The Phosphate Excretion Index (PEI)

PEI values above +0.09 were found in twelve out of sixteen patients with primary hyperparathyroidism, but also in several patients in the

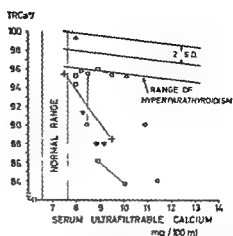


Fig 5 The tubular reabsorption of calcium (TRCa %) related to the concentration of ultrafiltrable calcium in serum. Thirteen patients with non-parathyroid hypercalcaemia. The following disorders are represented: ordinary hypercalcaemic sarcoidosis (○), postirradiation hyperparathyroidism over-treated by vitamin D (▽), idiopathic intestinal hyperabsorption hypercalcaemia (□), myelomatous (Δ), Hodgkin's paraneoplasia (+), and metabolic alkalosis and hypokalaemia (▲).

group of non-parathyroid hypercalcaemia. Some patients were examined at different CCr levels or on various admissions, which is indicated by a line between the PEI values in question. In the cases where CCr was below 55–65 ml/min, PEI was found to be inevitably raised, irrespective of the cause of the hypercalcaemia. Two patients (cases nos 23 and 28) with non-parathyroid hypercalcaemia had normal PEI at CCr above 70 ml/min but raised PEI at CCr below 50 ml/min (Fig 6).

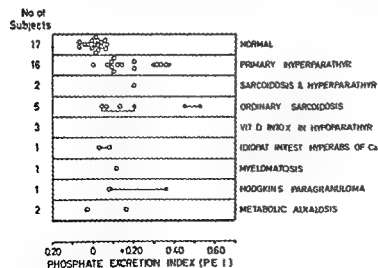


Fig 6 The 24-hour phosphate excretion index (PEI). Thirty-one patients with hypercalcaemia and seventeen normal subjects. The dashed lines represent the normal range (mean \pm 2 SD). It is indicated if the 24-hour clearance of creatinine is above (○) or below (●) 50 ml per minute.

DISCUSSION

The Interpretation of TRCa

It must be emphasized that the TRCa % values from different laboratories are not comparable. The results depend upon whether serum or plasma is used which part of the day the examination covers the intake of calcium and phosphorus (38) as well as the sodium intake (39) and the degree of renal insufficiency in the subjects studied (7).

Serum versus plasma

It has been shown by Hahneemann (23) that heparin is a calcium complexing agent. Accordingly serum UFCa is higher than plasma UFCa. This fact may explain why the 24 hour TRCa % values in our four normals 98.0-98.4% are slightly higher than in those of Kleeman et al (38).

Duration of the study

Laake (40) and Kleeman et al (38) found great variability in the TRCa % in normal subjects (92-99.5%) in clearance experiments of short duration while the variability was considerably less in 24 hour studies (38). In clearance experiments of short duration Kleeman et al (38) also found a remarkably lower TRCa % in primary hyperparathyroidism than has been found by any other investigator (11 21 22 40 41). An explanation for these differences is not immediately apparent. The most essential theoretical objection to the 24 hour TRCa % is that we do not know whether the used UFCa value is representative of the 24 hours. Carruthers et al found no diurnal variation in serum non protein bound calcium (13) while the observations regarding serum total calcium are contradictory (9 13). Despite the objection mentioned above the great variability of the TRCa in short term studies made the 24 hour determinations preferable.

Serum ultrafiltrable calcium

UFCa is essential both for the calculation and for the interpretation of TRCa % (38). When we use TRCa % instead of TRCa expressed in mg per minute we have corrected for one of the two parameters which determine the filtered load of calcium namely the glomerular filtration rate. Therefore the second parameter UFCa is the sole indicator of the filtered load of calcium. In

investigators who did not interpret the TRCa % in relation to UFCa (as exemplified in Fig. 2) arrived erroneously at the conclusion that PTH either does not affect or may even reduce the TRCa % (11 21 22 40). The demand for comparability with regard to the UFCa implies that the TRCa in hypercalcemic patients cannot be compared with the TRCa % in normal subjects.

The level of parathyroid activity

The effect of parathyroid hormone on TRCa % as it can be estimated from the observation of the TRCa % in primary hyperparathyroidism and in hypoparathyroidism has been the subject of much discussion. The most controversial finding has been a very high TRCa % in untreated hypoparathyroidism (21 22). The probable explanation is that parathyroid hormone only affects the reabsorption of calcium in the distal tubules. By sufficiently low filtered loads of calcium practically all filtered calcium is reabsorbed in the proximal tubules and the reduced parathyroid hormone secretion cannot manifest itself. Kleeman et al (38) contributed essentially to our knowledge of the effect of parathyroid hormone on TRCa % in man. Their most important findings were 1 that an increase in the filtered load of calcium decreased the TRCa % in all subjects and 2 that for any given filtered load of calcium the greater the level of parathyroid activity the higher the TRCa %.

The glomerular filtration rate

At clearance levels below 50 to 60 ml per minute the TRCa % decreases sharply in the group of non parathyroid hypercalcemia; this was not the case in primary hyperparathyroidism (Fig. 3). Our studies offer no explanation of this obvious difference. Better et al found that the TRCa % decreased with decreasing glomerular filtration rate in uremia, an observation which they attributed to a raised filtered load of sodium per nephron (7 8). The different effect of decreasing glomerular filtration rate on the TRCa % found in our two groups of hypercalcemias is however hard to explain in this way especially as the few available data on the renal sodium excretion showed no decisive difference between the two groups (Table I). It is important however to realize that the decreasing TRCa % represents a

way of escape from the rise in serum calcium concentration which would be a consequence of a lowered glomerular filtration rate this might be mediated by a suppression of the parathyroid glands. The lack of escape in primary hyperparathyroidism may be due to the autonomous non suppressible function of the parathyroid adenomas or primary hyperplasias.

A similar escape phenomenon with decreasing glomerular filtration rate is seen for phosphorus. But in contrast to the TRCa % the TRP % decreases (PEI increases) equally at low clearance levels in the two groups of hypercalcemias (see later). If the explanation of the escape phenomenon for calcium is true the escape phenomenon for phosphorus does not seem to be mediated through the parathyroid glands.

Hypercalcemic States Associated with Elevated Levels of TRCa %

Primary hyperparathyroidism

The normal 24-hour urinary calcium excretion in many of our patients corresponds to the findings of others (38, 56). It must be stressed that a normal urinary calcium excretion in a hypercalcemic patient must never be used as an argument against the diagnosis primary hyperparathyroidism—on the contrary! The high TRCa values and the decreasing tendency of TRCa with increasing UFCa (Fig. 4) agree with the conclusions of Kleeman et al. (38). Case no. 15 whose TRCa % was remarkably low 95.3% remained hypercalcemic (381 mg/24 h) during a normal calcium intake postoperatively although Ca^{++} was normalized. He is supposed to have had idiopathic hypercalcemia as well as primary hyperparathyroidism. Similar patients have been described previously (17, 22, p. 313).

Hyperparathyroidism associated with sarcoidosis

Case no. 17 is characterized as hyperparathyroid by presenting cortisone resistant hypercalcemia, parathyroid adenoma(s?) and an increased maximal tubular reabsorptive capacity for glucose (TmG/GFR) (25, 58). In good agreement with this the TRCa % was within the range of primary hyperparathyroidism (Fig. 4). In case no. 18 the assumption of hypersecretion of parathyroid hormone was supported by insufficient suppression of Ca^{++} during cortisone treatment, chief

cell hyperplasia of three parathyroid glands and an increased TmG/GFR. After parathyroidectomy Ca^{++} , TmG/GFR, TRCa %, and PEI were normalized (58). In spite of the evidence cited for parathyroid hyperfunction the preoperative TRCa % values were definitely reduced. Our present knowledge does not enable us to explain this finding.

Hypercalcemia associated with metabolic alkalosis and hypokalemia

The combination of hypercalcemia and normocalcemia is frequently found in the so-called milk alkali syndrome (10). A raised calcium intake is usually considered to be mainly accountable for the hypercalcemia but instances where the syndrome developed during high alkali—normal calcium—intake have been described (for review Punsar and Somer (47)). Although marked hypercalcemia may occur in patients with considerably reduced CCr caused by ordinary hypercalcemic sarcoidosis (Table I, cases no. 19 and 20) the normocalcemia in the milk alkali syndrome is generally attributed to renal insufficiency. Ingestion of alkali reduces the urinary calcium excretion in normal subjects (15) and in hypercalcemic sarcoidosis. In the latter condition it may even accentuate the hypercalcemia (43). Despite this fact no special attention has been paid to the alkalosis.

Both our patients who were studied during normal calcium intake were characterized by metabolic alkalosis, hypokalemia and low urinary calcium excretion despite hypercalcemia and practically normal CCr. In spite of the presumed suppression of parathyroid hormone secretion the TRCa % was even higher than in any case of primary hyperparathyroidism (Fig. 5). This must be attributed to a specific effect of the electrolyte disturbance on the TRCa. The fact that induced metabolic acidosis (which is associated with a considerable potassium depletion) reduces TRCa and tends to reduce UFCa in normal man indicates the importance of the alkalosis (Transbøl, Hahnemann and Thomsen, unpublished). On the other hand the presence of a nearly comparable alkalosis in cases no. 18, 23 and 24 which had no hypokalemia had no apparent effect on TRCa (Table I). If anything this might be taken as an indication that hypokalemia is of primary importance. Unfortunately

it is very difficult to study separately the individual roles played by alkalosis and hypokalemia. Both metabolic alkalosis and potassium depletion are common features of the milk alkali syndrome; thus our observations implicate that an increased tubular reabsorption of calcium may contribute to the development and maintenance of hypercalcemia in the milk alkali syndrome. It follows that TRCa % cannot be used for the differentiation between primary hyperparathyroidism and the condition in question.

Hypercalcemic States Associated with Depressed Levels of TRCa %

In agreement with the well established interplay between the parathyroid hormone secretion and the Ca^{++} , a functional hypoparathyroidism leading to a depression of TRCa % must be expected in non parathyroid hypercalcemia (the milk alkali syndrome excepted) (57). It has been found in normal man that hypercalcemia caused by calcium infusion reduces the TRCa % (4, 38) and in animals Sherwood et al (51) showed that induced hypercalcemia inhibited the secretion of parathyroid hormone. The combination of these observations affords the first experimental confirmation of the basic idea in the present work.

Poststrumectomy hypoparathyroidism overdosed with vitamin D

All three patients presented moderate hypercalcemia, reduced CCr, hypercalcemia and considerably decreased TRCa % as also described by Canary et al (12). When increasing the concentration of calcium in serum in a patient with untreated hypoparathyroidism hypercalcemia and reduction of the TRCa % occurs even before normocalcemia is obtained. This takes place during i.v. calcium infusion (4, 16) as well as during vitamin D treatment (4, 21, 22, 38) and thus it cannot be attributed to any specific effect of vitamin D on the tubular reabsorption of calcium.

Ordinary hypercalcemic sarcoidosis

The disturbance of calcium metabolism in sarcoidosis is attributed to intestinal hyperabsorption of calcium (1, 28). As hypercalcemia with or without elevation of the serum total calcium concentration may occur in spite of normoabsorption it has been claimed that sarcoidosis may

exert a specific effect on the TRCa % or on the bones (12, 27, 35). However, liberation of calcium from metastatic calcifications may also be responsible (32). Calcium balance and tracer studies revealed hyperabsorption of calcium in cases no 19 and 22 while in case no 23 where the patient was examined in the course of a spontaneous remission absorption was normal (32). All five patients had reduced TRCa %, analogous with the observations of Canary et al (12) and also decreased tubular reabsorption of glucose as previously mentioned (58). The latter finding agrees with our assumption that the decrease in the TRCa % is due to functional hypoparathyroidism (57). Hypercalcemia in presence of a normal concentration of total calcium in serum may be explained in the same way as Ca^{++} in sarcoidosis frequently is found raised with normal total calcium concentrations (24). The complex bound serum calcium fraction is also frequently raised in hypercalcemic sarcoidosis (24) but as this is also the case in some conditions with increased TRCa % the importance of the complex bound fraction for the tubular reabsorption of calcium is not clarified.

Idiopathic intestinal hyperabsorption hypercalcemia

This newly recognized entity is characterized by cortisone resistant intestinal hyperabsorption of calcium - hypercalcemia, normophosphatemia, normal phosphate excretion index, excessive hypercalcemia, depressed TRCa % and TmG/GFR - ratio associated with histologically normal parathyroid glands. Probably it represents an extreme variant of that type of idiopathic hypercalcemia which is attributed to a primary intestinal hyperabsorption of calcium (33). The low TRCa % is believed to be due to suppression of the parathyroid hormone secretion. A similar patient (subject A.W.) has been followed since 1953 by Pyrah (48) and subsequently by Hodgkinson and Edwards (31).

Hypercalcemia associated with Hodgkin's paraneoplasia

This disease resembles sarcoidosis with regard to clinical course as well as to immunology (37). In case no 28 the similarity also included the disturbance of calcium metabolism. The points of resemblance are 1) intestinal hyperabsorption of

calcium 2 a very variable hypercalcemia accentuated by exposition to sunlight and easily and completely suppressed by cortisone 3 normophosphatemia 4 reduced TRCa % (95.5%) and normal phosphate excretion index during border line hypercalcemia and 5 considerably reduced TRCa % (88.5%) and raised phosphate excretion index during a phase with moderate hypercalcemia and lowered CCr. On two occasions the TmG/GFR was surprisingly found to be slightly raised 2.19 and 2.44 (normal 1.87–2.07). As a small dose of prednisone (10 mg/24 h) has kept the serum calcium concentration normal for the last six months no indication for neck exploration has been present till now. As it is very temporarily group the patient as a case of non-parathyroid hypercalcemia but without neck exploration it cannot be decided whether he is in fact analogous to the patient with parathyroid hyperplasia and sarcoidosis (case no. 18).

Myelomatosis

The hypercalcemia in myelomatosis must be regarded as of non-parathyroid origin as it is due to a calcium releasing effect on bone by malignant plasma cells (2). In agreement with this a reduced TRCa % was demonstrated by Myers (44) and in the present study.

The Phosphate Excretion Index in Hypercalcemia

The phosphate excretion index was without differential diagnostic value (Fig. 6). This agrees well with the experiences of other investigators with the various parameters of phosphate excretion (44, 45, 56). If the tubular reabsorption of phosphate—and with that the P.E.I.—was mainly a function of the parathyroid hormone secretion reduced P.E.I. could be expected in patients with non-parathyroid hypercalcemia. However reduction of P.E.I. was never demonstrated. The causes for the inapplicability of P.E.I. with regard to differential diagnosis are 1 that irrespective of the cause of the hypercalcemia P.E.I. is raised at CCr levels below 55–65 ml/min (Table I and Fig. 6)—just as in renal insufficiency in general (19) 2 that the tubular reabsorption of phosphate is directly affected by the serum calcium concentration when the secretion of parathyroid hormone is reduced (16) and 3 that thyrocalcitonin seems to reduce the tubular reabsorption of phosphate (49).

Classification of Hypercalcemic Conditions According to the Functional State of the Parathyroid Glands

Only two parameters are necessary to classify the disorders of calcium metabolism in relation to the level of parathyroid activity. One is the concentration of ionized calcium in serum and the other a measure of the secretion of parathyroid hormone. When a disorder has been classified as hypercalcemic it would be preferable to measure the parathyroid activity by the concentration of parathyroid hormone in plasma or by the secretion rate of parathyroid hormone. When direct measurements of parathyroid hormone are unattainable an indirect measure may be relied on. The present study indicates that the TRCa % seems useful for this purpose. Other useable measures include the cortisone test and the maximal tubular reabsorptive capacity for glucose TmG/GFR (25, 58) while on the other hand the phosphate excretion index seems useless. Concerning the TRCa % it should be mentioned that none of our patients with primary hyperparathyroidism had such pronounced hypercalcaemia (600–700 mg/24 h) as Hodgkinson found in a few patients in his material (30). Therefore our observations have to be reexamined in larger groups of patients before the final differential diagnostic value of the TRCa % can be said to be established.

ACKNOWLEDGEMENTS

Supported by grants from the Danish Foundation for the Advancement of Medical Science and Statens almindelige Videnskabsfond.

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RENAL FUNCTION DURING CARDIAC PACEMAKING

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Abstract A hemodynamic and renal study was carried out on eight pacemaker treated patients with complete heart block. At the time of study the patients were normotensive and well compensated. Cardiac output, brachial arterial pressure, inulin and PAH-clearances as well as the excretion of electrolytes were determined on two or three selected heart rate levels between 45-118 for each patient during four to six 20 minute periods. A change of the ventricular rate from low to high level gave a significant decrease of stroke volume and pulse pressure but cardiac output, GFR and RPF were unchanged. The most interesting result was the finding of a significantly increased sodium excretion and sodium/potassium quotient on high heart rates. Possible explanations of the mechanisms responsible for this action are discussed.

Modern pacemaker treatment of patients with Adams-Stokes syndrome due to complete heart block has been a therapeutic success (8, 13, 15). This method has also offered new possibilities to study the response of the cardiovascular system in man to changes in the pacemaker induced ventricular heart rate both at rest and during exercise.

Such studies are of great interest not only in the individual patient in whom the ventricular heart rate giving the most favourable hemodynamic conditions should be aimed at but also in the physiological evaluation of the response in different sites of the peripheral vascular bed to changes in heart rate and cardiac output.

Several studies have dealt with central hemodynamic changes during cardiac pacemaking (4, 5, 6, 13, 14, 18, 19, 23) but comparatively few with renal splanchnic or cerebral blood flow (9, 11, 12, 24, 25).

This study was initiated in order to evaluate the effects in resting patients of changes in heart rate, blood pressure and cardiac output on renal hemodynamics and the renal handling of water

and electrolytes. This cardio-renal interrelationship is also believed to be of interest for understanding the mechanisms involved in the early development of congestive heart failure (1, 10, 26, 27).

MATERIAL

Eight patients with pacemaker-treated complete heart block were studied. The patients were all informed about the purpose of the investigation and willing to participate. Their clinical data are given in Table I. None of the patients had any history of renal or hypertensive disease. Patient 3 had a mild diabetes mellitus easily controlled by diet and without signs of glucosuria at the time of the study. Except for arteriosclerosis of variable degree no other previous or present disease of importance for this study was found among the aged patients. Three patients were on digitalis treatment as sign of congestive heart failure due to their heart disease had been noted, but at the time of the study all patients were well compensated and able to lie down flat during the three hours of the investigation without any cardiac discomfort. No other drugs were administered and no premedication was given before the study.

The pacemaker implantations had been performed from 5 to 10 days prior to the studies, using an endocardial electrode in the right ventricle and an external pacemaker (Elema-Schonander 138) for easy regulation of the heart rate.

METHODS

Indwelling vascular catheters were used for blood sampling, pressure recordings and infusions. Blood pressures were recorded by an Elema strain gauge manometer. Mean pressures were obtained by electrical integration. Heart rates were calculated from the ECG.

GloMERular filtration rate (GFR) and renal plasma flow (RPF) were estimated as the clearances of inulin and para-aminohippuric acid (PAH) using continuous infusion techniques and arterial blood sampling at the beginning and end of each period. Cardiac output was determined by a dye-dilution method using bromsulphalein (BSP) as indicator. An indwelling bladder catheter was

Table I Clinical data for the material at the time of study

Pat no	Sex	Age	Known history of complete heart block (months)	Heart volume (ml/m ² body surface)	Heart treatment	Serum creatinine (mg)
1	♀	73	14	520	Digitoxin 0.2 mg daily	1.1
2	♂	63	2	430	None	1.1
3	♂	70	2	600	Digitoxin 0.15 mg daily	0.9
4	♂	65	3	440	None	1.0
5	♂	58	5	540	None	0.7
6	♂	67	1	—	None	1.0
7	♂	73	4	570	Lanatoside C 0.5 mg daily	0.9
8	♂	56	—	480	None	0.8

used for urine collection and the bladder was rinsed with 100 ml distilled water and air. In two cases catheterisations were performed of a renal vein for the determination of the renal extraction of PAH. In these cases the PAH-concentration in blood was kept at a level of 5–6 mg in all other cases around 3 mg. The details of the methods and techniques used have been earlier reported from this laboratory (7).

Procedure

All studies were performed in the morning with the patients in the recumbent position. They were all in the fasting state and well hydrated. After the priming doses of inulin and IAH a continuous infusion of the clearance substances was started and 45 minutes were allowed for reaching equilibrium. During this time all patients were kept on a pacemaker rate of about seventy beats per minute. In all cases two clearance periods of 20 minutes were run on each selected heart rate level. In cases 1–5 the investigation started at a low pacemaker rate with a stepwise increase of the rate at the beginning of periods 3 and 5 so that in all six periods were run in succession. In cases 6–8 two equally long periods on a high rate were followed by another two on a low rate making four periods in all. Each patient was equipped with his own pacemaker battery allowing five fixed levels of heart rate varying however from one pacemaker to another. We accepted this variability of heart rate rather than disturb the patients' composure by changing pacemaker just before the study. The heart rate levels were chosen so that the patients felt comfortable and no signs of interference appeared. For these reasons the investigated frequency intervals are not of the same size in all patients.

The renal extraction of PAH was determined twice a period in cases 6 and 7.

Cardiac output was determined at the beginning of the second period at each selected heart rate level. In case 8 however cardiac output was not determined due to a technical failure. Brachial arterial blood pressures were registered 2–3 times in each period and immediately before and after the determinations of cardiac output. Continuous ECG registration was used.

Calculations

Renal clearances, renal blood flow, cardiac output as well as the renal fraction of cardiac output were calculated according to standard formulae (12). Vascular resistances are given as arbitrary units. The mean values of all observations made during the two clearance periods on each heart rate level were used in the calculations except for the calculation of the systemic vascular resistance in which the pressures registered immediately before and after the determination of cardiac output were used. In cases 6 and 7 the figures determined for the renal extraction of PAH were used in the calculations of the renal blood flow and renal fraction of cardiac output. In the other cases the PAH-extraction was assumed to be 90%. The renal venous pressure was always set to zero as none of the patients had clinical signs of elevated systemic venous pressure.

Differences between mean values for the first and the last two periods for each parameter were analysed by T test.

We considered this method justified although the heart rate levels for the patients were not identical as it can not be assumed that identical heart rates give corresponding hemodynamic or renal function in these patients with different heart size and myocardial condition. The changes were calculated in per cent for each parameter except for systemic vascular resistance, renal resistance, renal fraction of cardiac output and Na/K quotient.

RESULTS

Fig 1 shows the findings in one representative case (no 3) and data for all the material are given in Tables II and III with statistics. In cases 6–8 the sequence of the clearance periods is reversed in the tables in order to facilitate the comparison of data from low to high heart rate levels with the other cases.

All patients were within fairly normal ranges of arterial blood pressure with regard to their

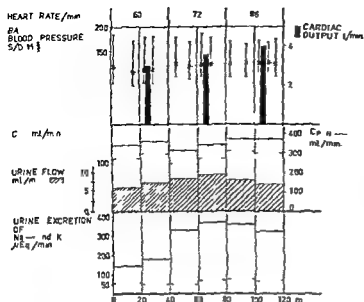


Fig 1 The basal hemodynamic and renal findings in case 3. Two clearance periods were run on each selected heart rate of 60, 72, and 86 beats per minute.

ages. The cardiac output was slightly reduced when studied at the lowest heart rate levels. GFR and RPF were subnormal in all patients except

nos 3 and 5. The mean value of the lowest heart rate level for the whole group was 56 and of the highest 96 beats per minute.

Table II Hemodynamic data for the material

In cases 6-8 the sequence of the clearance periods is reversed

Pat. no	Clearance period	Heart rate (b/min)	B P mm Hg		Cardiac output (l/min)	Stroke volume (ml)	Syst. vascular resist. (arb. units)	Hematocrit (%)
			Brach. art.	Mean				
1	1-2	48	186/74	110	2.9	61	37.2	41
	3-4	73	173/87	118	3.4	45	35.9	42
	5-6	80	170/96	122	3.1	35	39.7	42
2	1-2	68	133/81	104	3.9	60	26.7	38
	3-4	95	140/91	110	4.2	47	26.4	38
	5-6	118	138/95	109	4.1	34	25.9	38
3	1-2	60	178/81	109	3.0	51	36.7	40
	3-4	72	177/96	110	3.6	50	33.9	40
	5-6	86	168/95	123	4.0	46	30.8	39
4	1-2	45	152/73	93	4.1	91	22.6	38
	3-4	71	145/78	101	4.5	63	22.7	38
	5-6	86	152/85	104	4.6	65	22.8	39
5	1-2	48	167/81	105	4.5	58	23.6	44
	3-4	74	149/90	103	4.9	66	21.0	44
	5-6	107	147/96	105	4.9	56	21.4	44
6	3-4	68	161/85	105	3.7	54	28.1	42
	1-2	99	138/84	100	3.7	37	27.6	43
7	3-4	88	122/67	86	4.1	63	21.0	44
	1-2	100	173/75	81	3.8	4	24.5	44
8	3-4	48	156/79	115				
	1-2	105	141/91	110				
Mean change from lowest to highest heart rate			Pulse pressure					
			-25.9*		+8.4*	-34.3*	0.5	
S.E.			3.7		4.8	4.4	1.2	
P			<0.001			<0.001		

Table III Renal data for the material

In cases 6-8 the sequence of the clearance periods is reversed. Case 5 was excluded from the statistical calculations (for reasons see text)

Patient no	Clearance period	Heart rate (/min)	Urine flow (ml/min)	Inulin clearance (ml/min)	PAH clearance (ml/min)	Renal extraction of PAH (%)	Na (μ Eq/min)	K (μ Eq/min)	Na/K	Total renal resistance (arb units)	Renal fraction of cardiac output (%)
1	1-2	48	4.0	59	225	—	105	34	3.1	259	14.6
	3-4	75	3.7	70	277	—	176	41	4.3	222	15.6
	5-6	89	2.7	55	212	—	178	38	4.7	300	13.1
2	1-2	65	9.2	95	416	—	179	104	1.7	140	19.1
	3-4	95	7.6	88	398	—	192	91	2.1	154	17.0
	5-6	118	5.5	78	332	—	209	81	2.6	183	14.5
3	1-2	60	6.5	113	350	—	117	56	2.1	168	21.6
	3-4	7	8.6	103	327	—	355	58	5.6	198	16.8
	5-6	86	7.3	122	390	—	160	71	5.1	173	17.8
4	1-2	45	3.2	88	403	—	74	79	0.9	129	17.6
	3-4	71	4.2	91	436	—	118	70	1.7	139	17.4
	5-6	86	3.4	89	429	—	122	65	1.9	133	17.0
5	1-2	43	2.3	194	7.9	—	113	56	2.0	73	32.2
	3-4	74	4.1	127	45	—	91	52	1.8	115	18.3
	5-6	8	0.7	111	376	—	76	40	1.9	141	15.2
6	3-4	68	1.0	77	289	91	133	76	1.8	194	14.6
	1-2	99	2.9	84	203	92	140	75	1.9	172	15.7
	3-4	68	3.1	66	265	88	188	59	3.2	162	13.0
7	1-2	100	9.8	73	287	89	272	60	4.5	158	15.1
	3-4	48	1.9	96	463	—	140	81	1.7	—	—
	5-6	105	5.7	114	627	—	261	116	2.3	—	—
Mean change from lowest to highest heart rate			+52.3 /	+3.5	+5.8	—	+23	+6.9	+1.21	+11.2	-1.2
SE			41.3	4.7	6.3	—	27.4	8.5	0.35	10.5	1.08
P							<0.05				<0.01

Central hemodynamics

In all patients the ventricular heart rate was strictly conducted by the pacemaker activity at the rate levels selected and no interfering ventricular premature beats were observed. A change of the ventricular heart rate from low to high level was accompanied by a statistically significant narrowing of the pulse pressure averaging 26%. The mean arterial blood pressure did not change however. Cardiac output slightly increased in a few patients on increase of the heart rate from the lowest rate levels but this change was not statistically significant. The stroke volume consequently decreased. The systemic vascular resistance remained unchanged.

Renal effects

Large individual variations in the urine flow were observed but with no relation to the heart rate in the whole material.

GFR and RPF showed only minor changes with an unchanged filtration fraction. The total renal vascular resistance and the renal fraction of the cardiac output were similarly unchanged.

Sodium excretion increased significantly (72%) when heart rate changed from lowest to highest level. As potassium excretion remained unchanged the Na/K quotient significantly increased. In the two patients in whom the renal extraction of PAH was determined normal figures were found and no change was observed at different rate levels. In one patient (no 5) there was an obvious error in the renal clearance data giving suspiciously high figures for RPF and GFR in periods 1-4. This patient had a low and rapidly changing urine flow and the high figures for GFR and RPF are probably due to inadequate washout of the urinary tract. Whether or not this case was included in the statistical calculations the conclusions were the same. We did not include this case in our calculations of the renal parameters but report his mean data in order to keep to the sequence of the investigation.

DISCUSSION

Central hemodynamics

The idioventricular bradycardia of complete heart block is frequently accompanied by a low cardiac output (4, 25). Increasing the ventricular rate to 50-60 by ventricular pacing usually improves

the cardiac output significantly. A further increase however from 60-120 often does not augment the cardiac output at all or results in a small enlargement. Usually the most favorable cardiac output is found between 70-90 beats per minute. Sowton (23) found a correlation between such peaked curves and evidence of myocardial disease in the patients. When the myocardium is seriously damaged its ability to keep up a high cardiac output is reduced to a limited heart rate interval and outside this the cardiac output will fall.

In this study no significant change in cardiac output was found between the lowest and the highest heart rate levels. As the number of patients studied was rather small we did not attempt to correlate the cardiac output curves to the present signs of myocardial disease. Our findings seem however to be in good accordance with earlier reports showing above all that if there is a maximal cardiac output, it usually will be found between the heart rates of 70-90 beats per minute.

As cardiac output did not significantly change when the heart rate was increased there was a pronounced decrease in the stroke volume and pulse pressures. The mean arterial blood pressure and the calculated systemic vascular resistance showed only insignificant changes within the rate range studied.

Renal hemodynamics

The glomerular filtration rate and the renal plasma flow may increase with pacemaker treatment as compared to the situation with idioventricular bradycardia. This has been observed by Schuller et al. (20).

In this study the GFR and RPF remained unchanged when the ventricular heart rate was increased. This is in fact not surprising as the filtration pressure was found to be essentially unchanged. The variation in ventricular stroke volume and pulse pressure obviously had no influence on the GFR and RPF. In the two cases in which the renal extraction of PAH was determined no changes were observed indicating that the PAH-clearance is a valid measure of the renal plasma flow in this study. Individual variations in cardiac output at different heart rate levels were not accompanied by similar changes in renal blood flow.

Changes in ventricular stroke volume and pulse pressure obviously did not influence the renal hemodynamics suggesting an effective autoregulation mechanism. Our results are in agreement with those of Humphries et al (12) who noted unchanged GFR, RPF and cardiac output in pacemaker treated patients with complete heart block when changing their heart rate from 40–70 beats per minute.

The renal handling of water and electrolytes

The urine flow varied considerably in different patients and was not influenced by the heart rate. It is difficult in studies like this to achieve a constant hydration state as a rapid intravenous infusion might be hazardous to the circulatory state.

The most striking change found in this study was an increase of the renal excretion of sodium as well as the sodium/potassium quotient when the heart rate was increased. The increase of sodium excretion however did not correspond to the increase of heart rate as seen in cases 1, 2 and 4. Serum electrolytes were all unchanged during the studies (data not published) and as the GFR was also unchanged the increased sodium output could not be demonstrated to be due to an increase in the filtered load of sodium.

Dikler et al (9) observed a similar change in the sodium excretion in one patient with pacemaker when the heart rate was increased from 50–100 beats per minute. No simultaneous clearance studies were performed. Friedberg et al (11) studied the ability to excrete graded loads of sodium in four patients with total heart block before and after pacemaker treatment. They found a significant increase of the maximal excretion of sodium when the heart rate was returned to normal but no change in the glomerular filtration rate.

The increase in the Na/K quotient in this study raises the question whether the observed change has been mediated by an aldosterone mechanism. The decrease in ventricular stroke volume and arterial pulse pressure might possibly activate intrarenal receptors and the angiotensin-aldosterone system or even exert a direct influence on the adrenal cortex.

Although changes in the Na/K quotient throw suspicion on an altered aldosterone activity there

are certain arguments against such a mechanism in this study.

It is well known that there is always a delay of at least 30–60 minutes after the administration of aldosterone until the effect on electrolytes is observed even if the hormone is injected directly into one renal artery (3). Sharp et al observed (21) that exposure of a toad bladder to aldosterone for only five minutes followed by washing elicited a stimulation of the sodium transport reaching its maximum two hours after the washing procedure. It does not seem probable that a change in the aldosterone activity rate could account for the change in sodium excretion within 20 minutes as was found in this study whether the heart rate was changed from low to high or vice versa. A possibility remains that the changes in sodium output might be due to angiotensin which is presumed to exert a local action on the tubular reabsorption mechanism (16, 17).

Barger et al (2) showed that changes in the intrarenal distribution of blood can affect the excretion of sodium. An increased outer medullary flow was accompanied by a decrease in sodium excretion. Such changes in the renal blood distribution may occur without measurable changes in the GFR or RPF.

Whether changes in pulse pressure or stroke volume might affect the renin-angiotensin system or the intrarenal blood distribution via other intrarenal receptor systems is an interesting question that still remains to be solved.

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INTESTINAL ABSORPTION AND AUTOIMMUNITY IN ENDOCRINE DISORDERS

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Abstract Thirty-two patients with endocrine disorders were studied from gastroenterological and immunological points of view. Gastritis was found in 18 patients. It was atrophic in ten patients. Two of the latter had pernicious anemia with idiopathic hypoparathyroidism. Jejunal biopsy revealed a normal mucosa in 20 patients, an increase of "inflammatory cells" in four and villous alterations in six. Three of the latter had spontaneous hypothyroidism and two signs of autoimmune thyroiditis. A general malabsorption syndrome was present in eight patients; one had only steatorrhea and one possibly selective malabsorption of vitamin B₁₂. Of 19 patients with thyroid disease alone or in combination with other endocrine disorders, seven showed signs of malabsorption, six of whom also had villous alterations. Villous alterations were associated with immunological disturbances, except for one patient who had postoperative hypothyroidism. They did not respond to endocrine treatment nor to treatment with gluten-free diet, except for the patient without immunological changes. Distinct edema of muscularis mucosae was visible in 13 out of 16 patients with hypothyroidism. Malabsorption and villous changes in thyroid disease may be due to 1) myxedematous changes, 2) altered motility and 3) autoimmune phenomena. Of 12 patients with hypoparathyroidism alone or in combination with other endocrine disorders, four had signs of malabsorption, but only one with concomitant hypothyroidism and high thyroid antibody titers had villous alterations. Correction of calcium metabolism in three patients resulted in improvement of steatorrhea in all, but the one with concomitant thyroid disease. This may point to a purely functional relationship between absorption and calcium metabolism.

Histological and functional changes in the stomach and in the small intestine before and after treatment in hyperthyroidism have been described by the authors in previous communications (32, 33). The present report deals with gastrointestinal changes in other endocrine disorders, especially in hypothyroidism, autoimmune thyroiditis and hypoparathyroidism. Such a study seemed to be of considerable interest, firstly since alterations in

the endocrine functions may produce changes in the functional capacity of the gastrointestinal tract and secondly, since some endocrine diseases seem to have a common genetic and/or autoimmune background also postulated for some gastrointestinal disorders. It is the purpose of the present communication to show the occurrence of some intestinal changes, especially malabsorption in hypothyroidism, autoimmune thyroiditis and hypoparathyroidism and to discuss possible functional and autoimmune relationships.

MATERIAL AND METHODS

A number of patients admitted to the 1st, 2nd and 3rd Departments of Medicine, University of Helsinki during three consecutive years were studied. However, many patients refused the gastroenterological examinations and in several subjects the study had to be interrupted for various reasons. Altogether there remained 32 patients for the final analysis. The patients fall into several groups of endocrine disorders or combinations of them as shown in Table 1. Of the patients, 8 were females and 12 men. The mean age was 42 years (16-73 years).

The following gastroenterological examinations were carried out: gastric biopsy, 25 maximal histalog test, 4 biopsy of the small intestine, 30 determination of the fecal fat excretion according to the method of van de Kamer, 3, the vitamin A absorption test, 3, the d-xylose tolerance test, 7, and the Schilling test, 18.

The gastric and intestinal biopsies were taken with the Sclaff and the multipurpose suction biopsy tubes. In all but two patients biopsy samples were obtained from the proximal jejunum. In 5 patients 58 specimens were obtained from the body of the stomach. The biopsy specimens were fixed in 10% neutral formalin and stained with hematoxylin-eosin and PAS.

General immune responses were looked for by the aid of conventional paper electrophoresis and immune electrophoresis of the serum proteins. Circulating thyroid antibodies were tested for with the red tanned cell hemagglutination and the complement fixation technique. Routinely

Table II Some data on the patients with endocrine disease associated with pernicious anemia and malabsorption

Pat	Sex	Age	Endocrine disease	Co-existing diseases	Gastroenterological findings		
					Gastric mucosa	Intestinal mucosa	Signs of malabsorption
T G	♀	61	Spontaneous hypothyroidism	—	Normal	Partial villous atrophy (Fig. 1)	Only Schilling 1 and 11 pathological
S V	♂	62	Spontaneous hypothyroidism	—	Lymphocytic infiltration normal glands	Subtotal villous atrophy	Diarrhea fecal fat 29 g/day vitamin A and xylitol and Schilling tests pathological
P M	♀	16	Spontaneous hypothyroidism	—	Not examined	Subtotal villous atrophy	Diarrhea vitamin A and xylitol tests pathological
A P	♀	24	Postoperative hypothyroidism	—	Moderate atrophic gastritis	Subtotal villous atrophy (Fig. 2)	Diarrhea fecal fat 18 g/day vitamin A and xylitol tests pathological
O B	♂	45	Postoperative hypothyroidism	—	Not examined	Granulomatous ileo-colitis	Diarrhea fecal fat 9 g/day vitamin A and xylitol tolerance tests pathological
K K	♀	47	Postoperative hypothyroidism and hypoparathyroidism	—	Normal	Subtotal villous atrophy (Fig. 3)	Diarrhea fecal fat 19 g/day vitamin A and xylitol tolerance tests pathological
O J	♀	41	Asymptomatic thyroiditis	—	Normal	Subtotal villous atrophy	Diarrhea fecal fat 12 g/day vitamin A and xylitol tolerance tests pathological
E E	♀	45	Idiopathic hypoparathyroidism	Pernicious anemia diabetes	Gastric atrophy	Normal (Fig. 7)	Diarrhea fecal fat 33 g/day vitamin A and xylitol tolerance tests pathological
M N	♂	32	Idiopathic hypoparathyroidism	Pernicious anemia	Gastric atrophy	Normal	No
L G	♂	62	Postoperative hypoparathyroidism	Waldenström's macroglobulinemia	Moderate atrophic gastritis with metaplasia	Normal	Diarrhea fecal fat 18 g/day vitamin A and xylitol and FGLU tests pathological
H H	♀	51	Parathyroid adenoma with Addison's disease	—	Not examined	Normal	Diarrhea fecal fat 23 g/day no other signs of malabsorption

changes were seen only in 2 out of 16 patients with other endocrine diseases. In two hypothyroid patients in which the biopsy was repeated after adequate substitution therapy the edema disappeared. No PAS positive material was observed in the muscularis mucosae contrary to the findings of Douglass and Jacobson (9) in necropsy specimens from myxedematous patients.

Biopsy of the small intestine revealed subtotal villous atrophy (Figs 2 and 5) in five patients and partial villous atrophy (Fig. 1) in one subject. The distribution of these patients within the different groups is of some interest: one patient

had asymptomatic autoimmune thyroiditis, three had spontaneous hypothyroidism (one of them with high thyroid antibody titers and one with cytologically evident thyroiditis), one had postoperative hypoparathyroidism and hypothyroidism with high thyroid antibody titers (K. K.) and only one had pure postoperative hypothyroidism without any signs of autoimmune thyroiditis. All six patients also had an increased number of plasma cells and lymphocytes in the intestinal mucosa (Figs 2 and 5). Three additional patients with hypothyroidism showed an increase of these cells without epithelial changes (Fig. 6).

of the small intestine to treatment		
Endocrine	Gluten free diet	Comments
Normal	No	Presumably selective vitamin B ₁₂ malabsorption still under investigation partial villous atrophy not responding to treatment
Treatment still in progress	Symptomatic only	Malabsorption and thyroid antibodies found in 1963 hypothyroidism developed in 1967 poor response to gluten free diet elevated thyroid antibodies
Treatment still in progress	Treatment still in progress	Malabsorption with subtotal villous atrophy the follow up still in progress biopsy lymphocytic thyroiditis
Normalization of intestinal mucosa (fig. 4) and absorption tests	No	Malabsorption with villous atrophy responding to endocrine treatment during normal diet
	Not examined	Died of intestinal obstruction autopsy granulomatous enterocolitis
	No	Malabsorption with villous atrophy not responding to any treatment elevated thyroid antibodies
Normalization of absorption tests	No	Malabsorption with villous atrophy not responding to gluten free diet elevated thyroid antibodies
	No definite effect	Began in early childhood as hypoparathyroidism malabsorption pernicious anemia and diabetes developed later on distinct malabsorption without morphological alteration responding to treatment of hypoparathyroidism
	Not examined	Described in detail by Ikkala et al (14)
Disappearance steatorrhea	Not examined	Malabsorption with a normal intestinal mucosa associated with Waldenström's macroglobulinemia and postoperative hypoparathyroidism disappearance of steatorrhea during endocrine treatment with a normal diet
Not followed up	Not examined	Steatorrhea without other signs of malabsorption with normal intestinal mucosa and normal pancreatic function

and one patient was found on operation to have granulomatous ileo-colitis. Altogether 10 out of 32 patients had some anatomical changes in the small intestine.

Closer examination of the intestinal biopsy specimens revealed normal amounts of Paneth cells in all specimens and no PAS-positive material or lymphangiectasiae in the lamina propria. Generally no qualitative alterations were evident in the various specimens with villous changes. In addition no essential differences could be demonstrated on comparison with specimens taken

from patients with so-called gluten-enteropathy not associated with endocrine diseases.

Some data on the patients with malabsorption and with pernicious anemia are compiled in Table II. Absorption studies disclosed malabsorption in ten patients six of them had also changes in the structure of the villi and one had granulomatous ileo-colitis. In eight patients there was a general malabsorptive pattern with steatorrhea one patient had only steatorrhea (H H) and one (T G hypothyroidism) only a pathological absorption of vitamin B₁₂ in the Schilling I and II tests. It



Fig 2 Patient A P with postoperative hypothyroidism before treatment Subtotal villous atrophy with proliferation of the crypts and increase of the cell content in the lamina propria H.E. $\times 75$



Fig 3 Patient A P with postoperative hypothyroidism before treatment Edema and thickening of muscularis mucosae visible H.E. $\times 25$



Fig 4 Patient A P with postoperative hypothyroidism after adequate substitution therapy The mucosa including muscularis mucosae appears almost normal H.E. $\times 100$

seemed that signs of malabsorption were often associated with spontaneous hypothyroidism and idiopathic hypoparathyroidism occurring in four out of eight such patients Malabsorption was however also observed in four other patients but all these had also some thyroid or parathyroid disturbances

Clinical signs of chronic pancreatitis were not observed The secretin test was carried out only in five patients with steatorrhea the bicarbonate concentration was normal in three and slightly decreased in two subjects

Of the ten patients with steatorrhea six were re studied after correction of the endocrine failure The fecal fat excretion became normal in three of the six patients (one with postoperative hypothyroidism one with postoperative hypoparathyroidism and Waldenström's macroglobulinemia and one with idiopathic hypoparathyroidism) The remaining three patients who showed no response had some thyroid disorder (one had spontaneous hypothyroidism one postoperative hypoparathyroidism)

Table III Immunological features of patients with endocrine diseases associated with malabsorption and pernicious anemia

Pat.	Endocrine disease	Gastrointestinal signs	Serum immunoglobulins	Thyroid antibodies	Intrinsic factor antibodies	Not organ specific antibodies	Antibodies against whole human gastric mucosa
T □	Spontaneous hypothyroidism	Partial villous atrophy malabsorption of vitamin B ₁₂ normal gastric mucosa	Increase	Absent	Present	Absent	Absent
S V	Spontaneous hypothyroidism	Subtotal villous atrophy malabsorption lymphocytic infiltration of the gastric mucosa	Increase ^b	Present TRC 1 2 5 mill cf 1 64	Not examined	Not examined	Not examined
P M	Spontaneous hypothyroidism with lymphoid cell thyroiditis	Subtotal villous atrophy malabsorption	Decrease	Absent	Not examined	Not examined	Not examined
A P	Postoperative hypothyroidism	Subtotal villous atrophy malabsorption atrophic gastritis	Increase	Absent	Present	Absent	Absent
G B	Postoperative hypothyroidism	Granulomatous enterocolitis malabsorption	Increase	Not examined	Not examined	Not examined	Not examined
K K	Postoperative hypothyroidism and hypoparathyroidism	Subtotal villous atrophy malabsorption normal gastric mucosa	Increase	Present TRC 1 2 5 mill	Present (?)	Absent	Absent
O J	Asymptomatic thyroiditis	Subtotal villous atrophy malabsorption normal gastric mucosa	Increase	Present TRC 1 2 5 mill cf 1 4	Not examined	Present	Present
E E	Idiopathic hypoparathyroidism diabetes	Pernicious anemia with gastric atrophy malabsorption normal jejunal mucosa	Normal	Absent	Present	Absent	Absent
M □	Idiopathic hypoparathyroidism	Pernicious anemia and gastric atrophy	Not examined	Not examined	Not examined	Absent	Absent

□ Fine needle biopsy lymphocytic thyroiditis

^b Normalized after treatment with thyroid hormones

roidism and hypothyroidism with high antibody titers (K. K.) and one asymptomatic autoimmune thyroiditis. Additional trials with gluten free diet in these three patients did not improve the steatorrhea.

The changes in the intestinal villi were studied in four patients after periods of from four months to two years of substitution therapy for the endocrine disease. In only one patient the villous changes disappeared (A. P., postoperative hypothyroidism) (Figs 2, 3, 4). In the remaining three patients there was also no change in response to a glutenfree diet regimen. All three patients seemed to have normal amounts of Paneth cells. All patients were thyroid cases (Table II).

The results from the immunological examinations are compiled in Table III. The studies were carried out in the patients listed in Table II, i.e. those with morphological changes in the small

intestine and with pernicious anemia. An increase of the immunoglobulins was found in 6 and a decrease in one out of 8 patients. Thyroid antibodies were present in the blood of three patients (one had asymptomatic thyroiditis, one spontaneous hypothyroidism and one postoperative hypoparathyroidism) and hypothyroidism with high thyroid antibody titers (K. K.). Intrinsic factor antibodies were found in the sera of 2 out of 4 patients. In one of the patients (K. K.) the result was not conclusive. Parietal cell antibodies were detected only in the serum of a patient with asymptomatic thyroiditis (O. J.). Antibodies which were not organ specific (antinuclear factor, positive Waaler-Rose and latex fixation tests) were present only in the serum of this patient. Also gastric body mucosa antibodies were only found in this patient.

From the tables it appears that out of the 19

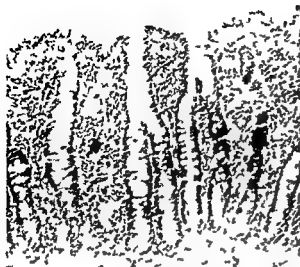


Fig 2 Patient A P with postoperative hypothyroidism before treatment. Subtotal villous atrophy with proliferation of the crypts and increase of the cell content in the lamina propria. H E $\times 75$.



Fig 3 Patient A P with postoperative hypothyroidism before treatment. Edema and thickening of muscularis mucosae visible. H E $\times 275$.



Fig 4 Patient A P with postoperative hypothyroidism after adequate substitution therapy. The mucosa including muscularis mucosae appears almost normal. H E $\times 100$.

seemed that signs of malabsorption were often associated with spontaneous hypothyroidism and idiopathic hypoparathyroidism occurring in four out of eight such patients. Malabsorption was however also observed in four other patients but all these had also some thyroid or parathyroid disturbances.

Clinical signs of chronic pancreatitis were not observed. The secretin test was carried out only in five patients with steatorrhea; the bicarbonate concentration was normal in three and slightly decreased in two subjects.

Of the ten patients with steatorrhea six were re-studied after correction of the endocrine failure. The fecal fat excretion became normal in three of the six patients (one with postoperative hypothyroidism, one with postoperative hypoparathyroidism and Waldenström's macroglobulinemia and one with idiopathic hypoparathyroidism). The remaining three patients who showed no response had some thyroid disorder (one had spontaneous hypothyroidism, one postoperative hypoparathy-

was seen. The only patient who did not respond was the patient with concomitant postoperative hypothyroidism and high thyroid antibody titres (K. K.).

No signs of malabsorption were observed in other endocrine diseases (hypopituitarism Cushing's syndrome and Addison's disease).

DISCUSSION

The association of various thyroid diseases (autoimmune thyroiditis, hypothyroidism and hyperthyroidism), Addison's disease, hypoparathyroidism, pernicious anemia, atrophic gastritis and possibly diabetes mellitus is presently of our standing interest. In thyroid diseases gastritis (17, 32, 33), pernicious anemia (8, 16, 18, 31, 34, 35), Addison's disease (4, 5, 15) and diabetes mellitus (1, 2, 5, 21, 22) are more prevalent than in the normal population. Antibodies against parietal cells of the stomach and intrinsic factor (8, 10, 17, 18, 31, 37) and adrenal tissue (15, 19) are more common findings than in the average population. A common, possibly genetically determined autoimmune background has been suggested (19). The same applies to idiopathic hypoparathyroidism in which there seems to be some association with pernicious anemia and Addison's disease (3, 13, 14, 24). The present series of 32 patients with various endocrine diseases in various combinations may hence be of some interest.

Gastric changes

The common occurrence of gastritis (70% of the cases) is also of some interest since the gastritis was atrophic in 40% (10 patients). Pernicious anemia was present in three of those ten patients and was associated either with spontaneous hypothyroidism or idiopathic hypoparathyroidism. One of the cases in the last mentioned group (M. N.) has been previously reported (14). In these cases adequate correction of the calcium metabolism did not influence the absorption of vitamin B₁₂. It is hardly conceivable therefore that the alterations in calcium metabolism induced vitamin B₁₂ malabsorption. A common background for these two conditions may therefore be postulated. Furthermore the simultaneous occurrence of idiopathic hypoparathyroidism, pernicious anemia, intestinal malabsorption and diabetes mellitus in

one case (E. E.) rather points in the same direction.

Small intestine

Less is known about the relationships between endocrine disorders and the function of the small intestine. Signs of malabsorption were observed in ten patients including one patient possibly with selective malabsorption of vitamin B₁₂ (T. G.). In six of these patients morphological changes in the intestinal villi were observed in one patient the changes were only slight. In the remaining four all of whom suffered from parathyroid disease the structure of the villi was entirely normal.

Of the 19 patients with thyroid disease seven showed signs of malabsorption. It might be significant as already mentioned under the Results that in four of these patients (S. V. P. M. K. K. and O. J.) there were signs of autoimmune thyroiditis that one had spontaneous hypothyroidism most probably due to autoimmune thyroiditis although no circulating antibodies were detected. Of the remaining 14 patients only two with postoperative hypothyroidism had signs of malabsorption, one of them also had granulomatous enterocolitis. In five of the seven patients with malabsorption the intestinal biopsy showed subtotal villous atrophy (S. V. P. M. A. P. K. K. and O. J.) four of whom had signs of autoimmune thyroiditis and one pure postoperative hypothyroidism. Partial villous atrophy was seen in one case (T. G.) with spontaneous hypothyroidism and vitamin B₁₂ malabsorption and one (G. B.) had granulomatous enterocolitis.

Unfortunately only a very limited number of patients could be adequately restudied after treatment. However the only patient in whom the malabsorption and the villous changes showed a definite response was one with pure postoperative hypothyroidism (A. P.) indicating perhaps that the changes may have been induced by "intestinal myxedema". In the three other patients there was no response either to treatment with thyroid hormones or to gluten free diet. Two of these had evidence of autoimmune thyroiditis (K. K. and O. J.) and one had spontaneous hypothyroidism (T. G.) with vitamin B₁₂ malabsorption.

According to the literature the association of malabsorption and hypothyroidism is uncommon.

Kelley and Stewart (23) described a patient with this combination and with intestinal hypomotility in which the signs of malabsorption did not respond to treatment with thyroid hormones. Salmi and Lahesmaa (27) drew attention to the common occurrence of gastrointestinal troubles in infantile hypothyroidism which they supposed to be due to myxedematous changes in the gastrointestinal tract. The possibility of myxedematous changes being the cause of various gastrointestinal disturbances is fairly acceptable in view of the myxedematous alterations observed in most patients with hypothyroidism in this series. But on the other hand one is apt to regard the prevalence of autoimmune manifestations in hypothyroidism accompanied by malabsorption and villous changes as being of significance.

Of the 11 patients with hypoparathyroidism three showed signs of malabsorption. The intestinal mucosa was abnormal only in one of them and this case had also hypoparathyroidism with high thyroid antibody titers (K. K.). When the endocrine disorder was adequately corrected the malabsorption disappeared in two patients but not in case K. K. This suggests that the malabsorption in the two cases without villous changes might have been in some obscure way due to the alterations in calcium metabolism.

The fairly common coexistence of malabsorption and idiopathic hypoparathyroidism has been stressed by several authors (6, 11, 20, 26, 28, 30, 36, 38). Jackson (20) believes that this combination is a definite syndrome although he was not able to offer a satisfactory explanation. Clarkson et al. (6) on the other hand are of the opinion that the steatorrhea is secondary to the endocrine disorder. In the patient they reported on the steatorrhea disappeared after adequate control of the calcium metabolism with vitamin D or parathyroid hormone. Similar experiences have been reported by others (24). This is well in keeping with the observations in the present series and also with that of Visakorpi and Gerber (36) in an infant with hypoparathyroidism, malabsorption and pernicious anemia. Morse et al. (26) suggested that the combination of malabsorption and hypoparathyroidism is only a variant of mucoviscidosis. These authors observed an increased sodium concentration in the sweat in one of five siblings with idiopathic hypoparathyroidism. Salvesen and Bøe (28, 30) have expressed the view

that the signs of hypoparathyroidism in steatorrhea may be due to the lack of some exogenous factor necessary for normal parathyroid function. In one of their patients *Candida albicans* was cultured in the feces and according to these authors this infection may have explained the presence of steatorrhea. Moniliasis is a common complication in this syndrome (29).

Malabsorption on the other hand is not common in postoperative hypoparathyroidism. In the two patients in the present series who had postoperative hypoparathyroidism and malabsorption other complicating conditions were also present. One had hypothyroidism with high thyroid antibody titers (K. K.) and the other had Waldenström's macroglobulinemia (L. G.) known to be associated with alterations in gastrointestinal functions. This would point to the view that the steatorrhea in idiopathic hypoparathyroidism is in some way associated with the endocrine disorder and may become manifest through the derangement of the calcium metabolism. This would suggest the existence of a real syndrome as suggested by Jackson (20).

Steatorrhea also occurred in a patient with primary hyperparathyroidism due to parathyroid adenoma and Addison's disease. The three other patients with Addison's disease in the present series had however no signs of malabsorption. It is not possible to offer an explanation of the malabsorption in this particular case.

General immunological phenomena were common in this series of patients with malabsorption. The immunoglobulins were increased in five patients, significantly elevated thyroid antibodies and cytologically proved autoimmune thyroiditis were found in four patients, intrinsic factor antibodies were observed in two out of four patients. The most striking pattern was seen in one patient with asymptomatic autoimmune thyroiditis (O. J.) who had elevated thyroid antibodies, positive latex fixation, gastric and nuclear antibodies, villous atrophy of the small intestine, some arthritic features and recurrent deep thromboses. This suggests that some autoimmune mechanism may be operative in the production of malabsorption and structural changes in the small intestine in association with some endocrine diseases. The increase of lymphocytes and plasma cells in the intestinal mucosa may also be of importance and may have some relation to the findings of Crabbe et al.

(7) according to which most intestinal plasma cells produce various immunoglobulins

On the evidence of the findings in the present series of patients it may be tentatively concluded that both changes in the endocrine functions and general autoimmune processes may be operative in the production of malabsorption. In hypothyroidism malabsorption and steatorrhea may be due to (a) myxedematous changes and associated metabolic alterations in the intestinal mucosa (reversible) (b) altered intestinal motility (reversible) and (c) general autoimmune phenomena (irreversible). In idiopathic hypoparathyroidism the steatorrhea which is not associated with morphological changes in the intestinal mucosa is corrected by adequate treatment of the changes in calcium metabolism. This may point to a purely functional relationship between the intestinal absorption and the calcium metabolism or the parathyroid hormone. It is of interest to note that no improvement of the malabsorption or of the villous changes occurred in response to gluten free diet in those patients who did not respond to endocrine correction in this respect. This suggests that gluten intolerance is usually not involved in this type of malabsorption associated with endocrine disorders.

ACKNOWLEDGEMENT

This study was aided by a grant from the Sigrd Juselius Foundation.

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VENTRICULAR ARREST CAUSED BY THE VALSALVA MANEUVER IN A PATIENT WITH ADAMS STOKES ATTACKS ACCOMPANYING DEFECATION

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Abstract A report is given of a 66-year-old man presenting a history of syncope accompanying defecation. In this patient atrio-ventricular conduction disturbances and vagal cardiosensitivity were noted. Performance of the Valsalva maneuver resembling straining at stool was associated with ventricular standstill and loss of consciousness. This observation suggests that pathophysiological changes similar to those produced by the maneuver were responsible for the attacks of syncope. It is postulated that myocardial hypoxia secondary to diminished blood pressure together with pronounced vagotonia were prime factors contributing to the asystole. Ventricular arrest has not previously been described as accompanying the Valsalva maneuver.

Certain respiratory maneuvers and even normal respiration affect the rhythm of the heart but no severe arrhythmias or conduction disturbances have been described accompanying the Valsalva maneuver. Syncope accompanying the maneuver and similar procedures such as straining at stool and cough usually are caused by the resultant drop in blood pressure. Below is presented a case showing that in a susceptible individual ventricular arrest induced by the maneuver may be the cause of syncope.

CASE REPORT

The patient, a 66-year-old man, was admitted on September 9 1964. In 1918 he suffered from severe influenza. In 1940 he had duodenal ulcer. For several years he had had low back pains. In 1958 he went through surgery for papilloma of the urinary bladder. Following a traffic accident in 1963 he complained of headaches however the syncopal seizures reported below occurred prior to this event.

For the last three years he suffered from fainting and loss of consciousness on exertion. In particular the attacks were induced by straining at stool and the patient would then assume the recumbent position as he had

learned that his symptoms were alleviated by this technique.

He was observed in the Department of Neurology in May 1964 and his symptoms were related to a possible intermittent insufficiency of the cerebral arteries. Evidence of encephalopathy and cerebral atrophy were present. Because of increasing frequency of the seizures he was later admitted to the local hospital (Innherred Sykehus Levanger Norway) where he had been observed previously and atrio-ventricular block grade I had been revealed. The patient now used isoprenaline tablets without any significant effect. Twice during that hospital stay he fainted when he had bowel movements. On one of these occasions he was observed by a nurse whose aid he had requested as he had felt uncomfortable and noted blurred vision. When she arrived the patient lost consciousness completely he was pale and the breathing became stertorous. He was pulseless for about fifteen seconds whereafter the pulse rate was 56/min irregular and the patient regained consciousness fairly soon. An electrocardiogram obtained at this point was unchanged. The episode described strengthened the suspicion that the attacks were of cardiac origin. As the patient suffered severely from the disease he was admitted to this department for further cardiac evaluation and treatment.

The patient complained of slight headaches and knee joint pains. Between the attacks he did not appear ill but his general demeanor suggested some degree of cerebral arteriosclerosis. On admission the pulse rate was 33/min regular BP 165/100 mm Hg. A systolic murmur grade III was heard at the apex. Otherwise the physical examination as well as routine urine and blood analyses were normal. The ECG showed a grade 2 atrio-ventricular block 2:1 and right bundle branch block (Fig. 1). Evaluation of the roentgenogram of the heart was unreliable since the patient had been unable to stand. Right carotid sinus stimulation resulted in advanced atrio-ventricular block grade 2 changing the 2:1 block into 3:1 block. Repeating of the procedure elicited transient atrio-ventricular block grade 3 and atrio-ventricular nodal rhythm. Left carotid sinus stimulation gave rise to atrio-ventricular nodal rhythm and numerous ectopic ventricular beats. No characteristic clinical symptoms occurred in association with these procedures. Rectal stim-

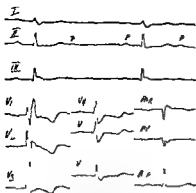


Fig 1 Admission electrocardiograms showing atrioventricular block grade 2 with 2:1 block and right bundle branch block

ulation inserting the gloved finger induced neither electrocardiographic changes nor clinical symptoms. The Valsalva maneuver performed with the patient in the supine position evoked no clinical or electrocardiographic changes. When the Valsalva maneuver was carried out with the patient sitting, further widening and deformation of the QRS-complexes were noted whereupon high grade atrio-ventricular block occurred in which successive atrial impulses were blocked resulting in ventricular asystole for several seconds. Some sinus acceleration took place simultaneously (Fig 2). The patient lost consciousness and the electrocardiographic recording had to be discontinued after a ventricular standstill of at least 46 sec. The Valsalva maneuver with the patient sitting was repeated after some days. Again high grade block was obtained resulting in a ventricular arrest of 35 sec but this time without concomitant syncope. Later the patient identified the symptoms preceding the loss of

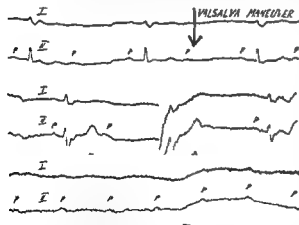


Fig 2 Electrocardiograms showing the effect of the Valsalva maneuver in the sitting position. Onset of the maneuver indicated by arrow. Consecutive recordings of standard Leads I and II demonstrating widening and deformation of the QRS-complexes, high grade atrio-ventricular block with blocking of successive atrial impulses, sinus acceleration and ventricular asystole.

consciousness induced by the maneuver with his spontaneous seizures. During the hospital stay he often complained of dizziness and attacks of faintness and once during the stay he experienced a short fainting spell accompanied by defecation.

On October 20 thoracotomy was performed and an artificial epicardial pacemaker applied. The immediate result was satisfactory. The heart rate was 75/min, regular as a result of parasystole (interference). Unfortunately the patient died suddenly and unexpectedly on the day after the operation. Autopsy did not reveal any findings that could explain the sudden death. Microscopic examination of the heart showed a subendocardial fibrous focus in the median wall of the right atrium.

DISCUSSION

The cause of the sudden death in this patient remains unknown. However, ventricular fibrillation induced by the pacemaker may very well have been responsible, since many predisposing factors were present: parasystole, early postoperative period, and parasympathetic tone (14). Constant supervision of such patients for some days after implantation of an artificial pacemaker seems important.

Defecation and digital rectal dilatation as well as may increase vagal tone and give rise to disturbances of the rhythm and conduction of the heart. In one case ventricular fibrillation was induced by rectal stimulation (12). There is no indication, however, that reflexes of this type were involved in the mechanism underlying the attacks of syncope accompanying defecation in our patient.

In the present case the Valsalva maneuver, simulating straining at stool, resulted in ventricular arrest and loss of consciousness. This observation indicates that pathophysiological changes similar to those produced by the maneuver were responsible for the syncopal attacks. The circulatory response to the Valsalva maneuver may be divided into four phases: 1 the onset of straining, 2 the period of sustained straining, 3 the period immediately after the release of straining, and 4 the subsequent period.

The normal response to the maneuver is as follows. During phase 1 a sudden increase in blood pressure occurs as straining empties blood from the lungs and the increased intrathoracic pressure is transmitted to the peripheral arterial vessels. This results in a reflex slowing of the heart rate. During phase 2 blood is prevented from entering the thoracic cage by the elevated intrathoracic pressure and as a result the cardiac

output falls and subsequently systemic blood pressure diminishes. Produced by sympathetic stimulation there will be an increase in the heart rate. In phase 3 with the sudden cessation of straining and loss of support of the intrathoracic pressure the blood pressure drops and the heart rate increases further. In phase 4 there is an overshoot in blood pressure and reflex bradycardia (4). Some normal individuals (3) and patients with cardiac disease in particular (4, 7) often fail to respond as outlined above. In our patient blood pressure was not measured during the maneuver but the sinus acceleration observed during phase 2 suggests that there was a normal response to the maneuver.

The development of the ventricular standstill during the Valsalva maneuver in this patient may be explained in the following ways:

(a) *Myocardial hypoxia*. Decreased blood pressure resulted in diminished coronary blood flow thus producing myocardial hypoxia which has a direct depressant action on the atrio-ventricular node ((2) p. 319) and ventricular pacemakers ((2) pp. 523-526).

(b) *Vagotonia*. Hypoxia increases the sensitivity of the vagal terminals of the heart in a constitutionally vagosensitive patient ((2) p. 526 (13)). In this patient pronounced vagal cardiosensitivity had been demonstrated and vagal stimulation induced by the hypoxia was critical. In addition the performance of the maneuver results in stimulation of the pulmonary stretch receptors initiating a vagal reflex ((2) p. 820).

(c) Following the asystole a further drop in blood pressure ensued thus giving rise to a vicious cycle.

The fact that the atrio-ventricular block was more marked in the sitting than in the recumbent posture may be explained by diminished cardiac output sitting accentuating the fall in blood pressure during the Valsalva maneuver. Also paradoxical postural mechanisms may have been present (11). The cerebral symptoms secondary to the circulatory failure may have been precipitated more readily sitting due to the decreased hydrostatic pressure in this position. The symptomatology possibly also was influenced by the cerebral atrophy noted.

Normal respiration and certain respiratory maneuvers affect the rhythm of the heart in various ways ((1) (2) p. 820) but no severe ar-

rhythmias or conduction disturbances have been described accompanying the Valsalva maneuver ((2) p. 820 (4, 6, 7, 9, 10)). However Bellet briefly mentions that cardiac arrest may be caused by the Valsalva maneuver following defecation ((2) p. 528). It is well established that the maneuver sometimes induces syncope due to the diminished blood pressure (3, 8, 10) which is believed to be the mechanism underlying the tussis syncope (5, 10). The present case suggests that in some individuals ventricular arrest may be the cause of syncope and even deaths following cough.

Vagal cardiosensitivity elicited by the carotid sinus reflex may possibly be used as a warning sign that sudden cardiac arrest or severe cardiac arrhythmias may occur in a susceptible individual under conditions that sensitize the vagal terminals in the heart ((2) p. 524 (13)). Since vagal tone increases during the Valsalva maneuver similar procedures such as straining at stool and vigorous coughing should be prevented as far as possible in these patients by proper measures and instructions.

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NECROTIZING VASCULITIS WITHOUT VISCERAL INVOLVEMENT

Postmortem Examination of Three Cases with Affection of Skeletal Muscles and Peripheral Nerves

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Abstract The biopsy and postmortem findings are reported in three elderly patients with a widespread necrotizing vasculitis of the small arteries and arterioles of muscles and peripheral nerves and no clear involvement of visceral organs. Most of the affected vessels had a diameter between 50 and 200 μ . Two of the cases also had a temporal arteritis and the third had a vasculitis of the small vessels surrounding the temporal artery. All cases showed fibre loss in the peripheral nerves probably caused by ischaemic nerve lesions and widespread denervation atrophy of the muscles. On the whole the nerves and muscles of the legs were most severely affected.

All cases had attacks of fever, high erythrocyte sedimentation rate and increased globulin fractions in the serum and all cases appeared to improve to some extent on steroid treatment. The clinical picture had features in common with temporal arteritis and polymyalgia rheumatica. It is emphasized that elderly patients with such symptoms may develop signs of neuropathy during the course of the disease which is due to a vasculitis of small vessels. This vasculitis may remain localized to muscles and peripheral nerves.

The present report summarizes the biopsy and postmortem findings in three elderly patients with a widespread necrotizing vasculitis of unknown cause which affected the small arteries and arterioles of muscles and peripheral nerves and largely left the visceral organs intact. Two of the patients also had a temporal arteritis. The intervals between the onset of symptoms and death were from 1 to 3 years. All cases received prolonged steroid treatment which may have modified the course of the disease and the findings at autopsy.

The main purpose of the report is to emphasize the following points: (a) In some cases of necrotizing angitis there may be a selective affection of certain organs or systems such as muscles and

peripheral nerves and little or no affection of visceral organs. (b) Temporal arteritis in elderly patients may be accompanied by a widespread vasculitis of small vessels. (c) There are intermediate types of necrotizing vasculitis which can not be classified into rigid subgroups.

CASE REPORTS

Case 1

In 1961 a 60 year old man developed rheumatic pains which were vaguely referred to the finger joints, shoulders and knees. He received gold injections and physiotherapy and apparently improved somewhat over the following months. In May 1964 he became worse with periods of fever, weight loss, malaise and pains throughout the body.

He was admitted to hospital for one month in September-October 1964. On admission he had a temperature of 39°C, severe and diffuse pains and tenderness over the tendon attachments in the neck. There were no signs of polyarthritides. The blood pressure was 140/65. The erythrocyte sedimentation rate was 16 mm per hour. White blood cell counts varied between 7400 and 11 000 per mm³. Differential counts were normal. Antistreptolysin and antistaphylolysin titres were within normal limits. Repeated urinalyses were unremarkable. LE cell preparations were negative. Total serum protein varied between 51 and 61 g per 100 ml. Electrophoresis of serum showed decreased albumin content (36% against normal 55-65%) and increase in α_2 globulin (9% against normal 3-5%), α_1 -globulin (19% against normal 4-8%) and γ -globulin (35% against normal 16-21%).

Steroid treatment was started shortly after admission (30 mg prednisone daily). Marked subjective improvement followed and the temperature fell rapidly to normal levels.

He was discharged on steroid treatment and remained fairly well for ~3 weeks. He then noticed increasing pains in both feet and calves and weakness of both legs. The symptoms progressed over a few days and he was readmitted to hospital in November 1964.



Fig 1 Inflammation in the wall of the temporal artery of case 1 Biopsy specimen Haematoxylin-eosin $\times 140$

On the second admission he had a temperature of 39 C. Both feet were almost paralytic with a severe hypoaesthesia up to the lower part of the calves. The left leg was somewhat more affected than the right.

After an increase of steroid doses to 60 mg daily the temperature again fell to normal levels and there was improvement in the general condition but no clear change in neurological symptoms.

Two weeks after the second admission and some days after reduction in steroid doses to 50 mg he again developed fever and severe pyrexia and hypoaesthesia of the right arm and slight paresis also of the left hand. There was some improvement after an increase of steroid doses to 100 mg daily and he was later largely afebrile. However the pareses persisted and he also continued to have pains. The erythrocyte sedimentation rate fell gradually during the course of the disease and stayed at 15 mm per hour during the last weeks. He died from a severe bronchopneumonia on February 2 1965.

Biopsies of the temporal artery and the soleus muscle

A biopsy specimen of the left temporal artery was taken in October 1964 (Fig 1). The lumen was nearly occluded by a thickened intima and the internal elastic membrane was fragmented. There was a subacute inflammatory process in all layers of the artery with a heavy accumulation of lymphocytes and histiocytes and a fair number of neutrophilic leucocytes. A platelet aggregate was attached to the intima. No multinuclear giant cells were observed. Two small vessels (700μ) in the connective tissue around the temporal artery showed severe inflammatory reaction and recent thrombi in the lumen.

A biopsy specimen of the left soleus muscle was taken in October 1964 three weeks after the onset of steroid therapy. A marked inflammatory reaction was seen in the walls of several small arteries with a diameter about 200μ (Fig 2). The lumen was occluded by fibrin thrombi. The vessel walls were partly necrotic with patches of amorphous material which stained like fibrin. The predominant inflammatory cells in and around the vessel walls were lymphocytes and histiocytes but there were also some neutrophilic leucocytes.

Postmortem examination

The cause of death was a severe bilateral bronchopneumonia. The large arteries showed a slight patchy atherosclerosis. The arteries of the mesentery and other small visceral vessels were grossly unremarkable. There was a small carcinoma with a diameter of 1.5 cm in the left kidney. Otherwise the kidneys and other visceral organs were unremarkable. No gross abnormalities were found in the brain or spinal cord.

Microscopical sections were examined from visceral organs, skin, brain, spinal cord and intraspinal nerve roots. In addition multiple sections were examined from muscles and peripheral nerves. These blocks were taken from the distal and proximal parts of the limbs and from the trunk.

The sections from the skin, brain, spinal cord, intraspinal nerve roots, spleen, heart, liver, kidneys and lungs showed no evidence of healed or active vasculitis. Two small arteries in the periadrenal adipose tissue and one artery between the muscle layers in the jejunum had thickened walls with a few lymphocytes in the media and adventitia. These three vessels were the only ones which could indicate a vasculitis in the visceral organs.

All examined muscles and nerves showed evidence of healed or slightly active vasculitis in arteries and arterioles (Fig 6). The affected vessels had a diameter between 25 and 400μ , the majority being from 50 to 700μ . The vessel walls were greatly thickened with subintimal connective tissue proliferation, patchy disruption of the internal elastic membrane and perivascular fibrosis. Many vessels also showed a fairly marked intramural and perivascular accumulation of lymphocytes. The lumen was often completely occluded, presumably by organized thrombi, or there was evidence of recanalization. Occasional perivascular lymphocytic aggregates were also found. There was no evidence of acute vasculitis.

The diseased nerve vessels were located both in the perineural connective tissue and within the nerve bundles. Both sciatic nerves showed severe patchy nerve fibre loss; some bundles were almost normal while neighbouring bundles contained very few fibres (Fig 3). In both tibial nerves only a few scattered myelinated fibres were left.

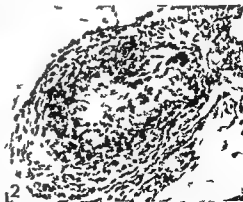


Fig 2 Inflammation and recent thrombus in a small muscle artery of case 1 Biopsy specimen Haematoxylin-eosin $\times 160$

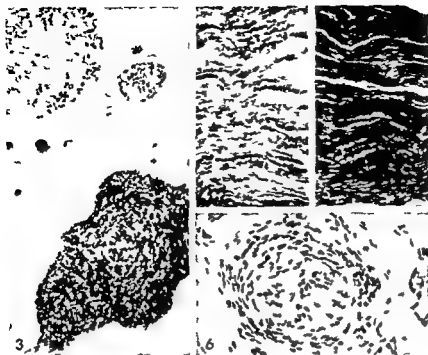


Fig 3 Two small nerve bundles with nerve fibre loss (top) and adjacent normal bundle (bottom) in the right sciatic nerve of case 1. Weolcke's myelin stain $\times 80$.

Figs 4 and 5 Abrupt transition from normal appearance (5) to Wallerian degeneration (4) in a nerve bundle from the left median nerve of case 1. Weolcke's myelin stain $\times 80$.

Fig 6 Small muscle artery with healed vasculitis from case 1. The lumen is almost occluded by connective tissue and there is slight perivascular chronic inflammation. Haematoxylin-eosin $\times 200$.

In a longitudinal section of the left median nerve a normal fibre bundle showed abrupt transition into Wallerian degeneration in the distal part of the section (Figs 4 and 5). It is therefore likely that the nerve fibre loss was due to ischaemic nerve lesions which were caused by occlusion of the small diseased arteries in the nerve.

All examined muscles showed varying degrees of denervation atrophy. This atrophy was most severe in the distal parts of the legs and relatively mild in the trunk muscles. No scars were found in the muscles after infarcts or other types of primary muscle destruction.

Several medium-sized and large arteries of the limbs and visceral organs were included in the sections. None of these showed any evidence of vasculitis.

Comment

This man who died at the age of 64 had a history of severe rheumatic pains three years before death which possibly was related to his terminal illness. Nine months before death he had diffuse pains throughout the body and fever. During the further course he had attacks of rapidly progressing asymmetrical pareses and hypoaesthesia in the legs and arms. The disease was accompanied by a strongly increased erythrocyte sedimentation rate, decreased serum albumin and increased serum globulins. There was a good clinical response to steroids but relapses in spite of heavy doses. Although there were only vague local symptoms a biopsy from the temporal ar-

tery showed a severe arteritis. A muscle biopsy showed an acute necrotizing vasculitis with thrombi in the small arteries and arterioles. Post mortem examination showed healed or slightly active vasculitis of the small arteries (50–200 μ) of all examined muscles and peripheral nerves. There was a severe patchy fibre loss in the peripheral nerves probably due to ischaemic nerve lesions and denervation atrophy of the muscles. The visceral organs showed little or no evidence of vasculitis.

Case 2

A previously healthy woman who died at the age of 76 had pains in the back and period of fever one year before death. Later the pains spread to the neck, shoulders, arms and calves. She was admitted to hospital in December 1965, three months after onset of the disease. On admission she had a blood pressure of 130/90. During the first week she had irregular fever up to 39°C. Fourteen days after admission she awoke one morning with a large central scotoma of the right eye which later remained unchanged. She also complained of numbness and coldness of the feet but no clear signs of neuropathy were found.

The erythrocyte sedimentation rate was 90 mm per hour. There was a slight normochromic anaemia. Leucocyte counts varied between 4700 and 19 600 per mm³. Differential counts were normal. Repeated urinalyses were negative. Total serum proteins were within normal limits. Electrophoresis of serum showed lowered albumin



Fig 7 Inflammation and recent thrombus in a small muscle artery from case 1. Biopsy specimen. Haematoxylin-eosin $\times 30$

(35%) increased β -globulin (3%) slightly increased β -globulin (18%) and normal α - and γ -globulins. LE cell preparations were negative.

Prednisone treatment (30 mg daily) was started on the day before the visual defect appeared. Her temperature fell rapidly to normal levels and there was a clear improvement in her condition. Later she had several new attacks of fever some of which occurred after attempts to reduce the steroid doses. She was discharged in a fairly good condition on steroid treatment. Moderate pains in the shoulders and legs persisted.

She was readmitted in July 1966 and stayed in hospital almost continuously until her death on October 3, 1966. The second admission was caused by a fairly acute exacerbation of pains in the back and thighs. Osteoporosis and compression fractures of several vertebrae were diagnosed. These vertebral lesions were considered partly responsible for her new symptoms. She was kept on steroid treatment and her symptoms remained largely unchanged until three weeks before death when she developed a pneumonia with septicæmia, shock and oliguria. She died from uraemia. The oliguria was probably caused by an acute tubular nephropathy precipitated by the shock and septicæmia.

Biopsies of the temporal artery and the m. pectoralis major were taken three weeks after the first admission. There were no local symptoms or signs of temporal arteritis (apart from the visual field defect). Histologically the temporal artery was unremarkable. However, several small arteries in the surrounding connective tissue showed vasculitis with necrosis of the walls, recent fibrin thrombi and intense inflammatory reaction with lymphocytes and some neutrophilic leucocytes. Similar changes were seen

in several small vessels in the p. toralis muscle (Fig. 7). The diameter of the affected vessels was about 100 μ .

Postmortem examination

Microscopic examination of the kidneys showed changes which were consistent with a tubular nephropathy caused by shock. There were no signs of vasculitis in the kidneys and the glomeruli were normal. There was a moderate pulmonary oedema. The heart was moderately hypertrophic and weighed 4.0 g. There was a small ulcer in the duodenum. The aorta and other large arteries showed a moderate atherosclerosis. The arteries of the mesentery and other visceral arteries were grossly normal. There was a severe osteoporosis of the spine with compression fractures of several thoracic and lumbar vertebrae.

Multiple blocks were taken for microscopic examination from many visceral organs, brain, spinal cord, peripheral nerves and muscles. No signs of vasculitis or other significant diseases were seen in the visceral organs or central nervous system. Evidence of healed or slightly active vasculitis was found in numerous small arteries and arterioles in all examined muscles and nerves (Fig. 11). The diameter of the affected vessels was between 50 and 100 μ . The lumen of the vessels was partly or completely occluded by connective tissue, sometimes with evidence of revascularization. The internal elastic membrane showed patchy disruption or splitting and there was frequently a marked perivascular concentric fibrosis. Slight to moderate lymphocytic accumulation was found in some of the vessels. No vessels showed acute vasculitis.

The affected vessels in the nerves were mainly located in the epineurium and perineurium but some were also found within the nerve bundles. Myelin stains showed a moderate patchy nerve fibre loss in the sural nerves and a severe fibre loss in the tibial nerves (Fig. 8). The fibre content of the median nerve was almost normal (Fig. 9). None of the examined nerves showed recent myelin breakdown. All examined muscles showed varying degrees of denervation atrophy (Fig. 10). The distal parts of the legs were most affected and the trunk muscles showed only slight atrophy. There was no evidence of healed infarcts in the muscles.

Comment

This 76-year-old woman had a one-year history of fever and pains throughout the body, but most severely in the back and legs. She had a strongly increased erythrocyte sedimentation rate, decreased serum albumin and increased α -globulins. Three months after the onset of the disease she suddenly developed a large central scotoma of the right eye which later persisted. There was a good clinical response to steroids, but several recurrences during the course. Biopsies from the temporal artery and the pectoralis muscle during the acute stage showed acute necrotizing vasculitis with recent thrombi in the small arteries and ar-



Figs 8 and 9 Nerve fibre loss in the left tibial nerve (8) from case 1 compared with a normal fibre content in the left median nerve (9) Woolcke's elin stain $\times 80$

Fig 10 Denervation atrophy of the left gastrocnemius muscle of case 2 Haematoxylin-eosin $\times 200$

Fig 11 Completely occluded small vessel in the left sciatic nerve from case 1 Haematoxylin-eosin $\times 400$

terioles in the muscle and in the connective tissue around the temporal artery but no temporal arteritis. On postmortem examination signs of healed vasculitis were found in numerous small arteries (50–200 μ) in the muscles and peripheral nerves but not in the visceral organs. There was a quite marked nerve fibre loss in the nerves of the legs probably caused by ischaemic nerve lesions and widespread denervation atrophy of the muscles. The distal muscles of the legs were most severely affected. Although information about neurological symptoms is lacking the marked morphological changes in the nerves and muscles strongly suggest that the patient had a significant neuropathy particularly in the legs.

Case 3

A 73-year-old man was admitted to hospital in 1965 with severe pain in the left side of the face and general malaise which had lasted for one week. His face appeared swollen in the region of the left maxilla and there was some tenderness around the left temporomandibular joint. The left temporal artery was thickened but not definitely tender. The temperature was 38°C. The erythrocyte sedimentation rate was 14 mm per hour. Serum electrophoresis showed increased α_2 globulin (16%). LE cell preparations were negative.

A biopsy of the left temporal artery showed a severe sub-acute inflammation with intramural a curtailation of lymphocytes, histiocytes and polymorphonuclear leucocytes (Fig 1). The internal elastic membrane was fragmented and there was a marked intimal connective tissue proliferation. There was a recent thrombus in the lumen. No multinuclear giant cells were seen. Several small ar



Fig 12 Inflammation in the wall of the temporal artery from case 3. Biopsy specimen. Haematoxylin-eosin $\times 170$

teries and one little vein in the surrounding connective tissue showed acute necrotizing vasculitis with recent thrombi.

Steroid therapy was started and he improved rapidly. He was discharged on steroids (7.5 mg prednisone daily) and apparently did not have any recurrences. No mention is made in the record about pain in other parts of the body or neurological symptoms.

Four years before the onset of the temporal arteritis he had been admitted to hospital because of hypertension and a moderate proteinuria which in retrospect probably was due to a chronic glomerulonephritis. He did not have any serious symptoms from his kidney disease until one year after the temporal arteritis when he developed a rapidly progressing uraemia and died after a few weeks illness.

Post mortem examination

The cause of death was a chronic membranous glomerulonephritis. There was no evidence of vasculitis in the kidneys. Nor were there any signs of necrotizing glomerulitis as has been described in hypersensitivity angitis (17). The heart weighed 570 g and showed myocardial fibrosis. The large arteries showed severe atherosclerosis. The brain and the spinal cord were grossly normal.

Blocks were taken for microscopic examination from visceral organs, muscles and peripheral nerves. None of the visceral organs showed any evidence of vasculitis.

There was a severe hyaline arteriosclerosis in the kidneys probably related to the hypertension.

The sciatic and tibial nerves from both legs were examined. Numerous small arteries and arterioles showed marked thickening of the walls with fibrosis and hyalinization of the media, subtotal or complete occlusion of the lumen and perivascular fibrosis (Fig 14). The diameter of the vessels varied between 50 and 200 μ . Most of them were found within the nerve bundles; others were located in the perineurium. No such changes were found in the nerves of the arms. A few small arteries were also found in the muscles which showed fibrosis of the media and complete or subtotal occlusion of the lumen by connective tissue (Fig 13). In some of them the internal elastic membrane was disrupted. None of the vessels in the nerves or muscles showed acute or chronic inflammation.

A moderate nerve fibre loss was found in the sciatic and tibial nerves (Fig 15) while the nerves of the arms appeared normal. All examined muscles from the legs showed quite marked denervation atrophy. Slight denervation atrophy was found in the muscles from the arms and trunk.

Since the changed vessels at autopsy in this case showed no inflammation it must be considered whether the thickened vessels in the nerves could be due either to a hypertensive arteriosclerosis or to some kind of obliteration which was secondary to the nerve fibre loss. However, the normal vessels in the nerves from the arms would seem to preclude a generalized hypertensive arteriosclerosis. Furthermore, the moderate nerve fibre loss could hardly cause the marked vascular changes. It is therefore likely that the vascular changes represent the end stage of a vasculitis and that the nerve fibre loss also in this case was due to ischaemic nerve lesions.

Comment

This 74-year-old man had a history of histologically verified temporal arteritis one year before death. Postmortem examination showed vascular changes in the nerves of both legs and a widespread denervation atrophy of the muscles which was most severe in the legs. Although information about relevant clinical symptoms is

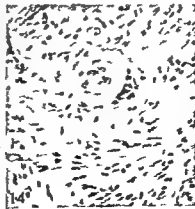
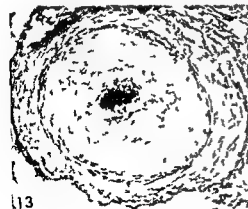
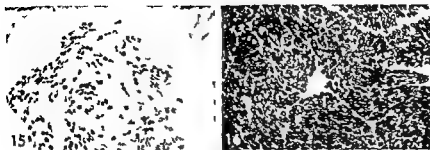


Fig 13 Subtotal occlusion by connective tissue and perivascular fibrosis of a muscle artery from case 3. Haematoxylin-eosin $\times 75$

Fig 14 Hyalinization of the media and proliferation of connective tissue in a small artery in the left sciatic nerve from case 3. Haematoxylin-eosin $\times 200$



Figs 15 and 16 Nerve fibre loss in the left tibial nerve (15) from case 3 compared with a normal fibre content of the left median nerve (16). Haematoxylin-eosin $\times 100$

lacking the morphological findings strongly suggest that also this patient had a vasculitis of the small vessels which had caused a widespread neuropathy.

The cause of death was a chronic glomerulonephritis which had caused symptoms 4 years before the onset of the arteritis.

DISCUSSION

From the biopsy and postmortem examinations it must be assumed that all three cases had a widespread necrotizing vasculitis which involved the small arteries and arterioles of muscles and peripheral nerves. Most of the affected vessels had a diameter between 50 and 200 μ . In addition cases 1 and 3 also had an arteritis of the temporal artery. Case 2 had a vasculitis of the small vessels around the temporal artery but no temporal arteritis.

Generalized necrotizing vasculitis of small vessels is known mainly to affect visceral organs although simultaneous involvement of nerves and muscles are frequent. The vasculitis of the present cases was remarkable in the lack of involvement of visceral organs. The only vague indication of visceral affection was seen in case 1 which showed a few thickened arteries in the periadrenal adipose tissue and in the intestine. These periadrenal vessels were close to nerve bundles and may in fact represent nerve affection. Necrotizing vasculitis may thus show a selective affinity for certain organs or systems. A similar case in which only the peripheral nerves were involved was described as polyarteritis nodosa by Kernohan and Woltman (9).

All three cases showed a clear nerve fibre loss in the peripheral nerves probably caused by occlusion of the small diseased vessels in the nerves. Varying degrees of denervation atrophy were

present in most examined muscles in all cases. This indicates a generalized nerve affection although there were marked quantitative differences in the various areas. Since the small vessels within the nerve bundles were affected the lesions sometimes involved only small parts of the cross section of the nerves (Fig. 3). Generally the distal parts of the limbs were most severely affected as could be expected from a summation of multiple small lesions along the course of the nerves.

Case 1 had several attacks of rapidly progressing asymmetrical paresis and hypoaesthesia of the limbs. Information about neuropathy is lacking in the two other cases. However the muscle atrophy and the nerve lesions of the legs were so severe that overt clinical symptoms probably were present also in these cases.

Although both muscles and nerves were affected by the vasculitis only the nerves showed evidence of ischaemic lesions. This may be due both to a better collateral circulation and to a greater tolerance to ischaemia in the muscles.

It is remarkable that in all cases the vascular lesions found at autopsy were either completely healed or showed only slight chronic inflammation. All cases received prolonged treatment with steroids and it is possible that this in part may be responsible for the uniform histological picture as has been claimed in steroid treated cases of polyarteritis nodosa (2, 20, 21). It should be noted however that relapses occurred even on very high steroid doses (90–100 mg daily).

Both clinically and pathologically the cases have features in common with several of the previously reported subgroups of necrotizing vasculitis. However they seem to represent intermediate types and do not quite fit into anyone particular group. Four relevant groups of disorders will be discussed briefly in this respect: 1. Polyarteritis nodosa proper, 2. hypersensitivity

angitis 3 temporal arteritis (giant cell arteritis) and 4 polymyalgia rheumatica which according to some authors may be caused by a giant cell arteritis (1 3 4 6 13 15 16 19 22)

It is not yet clear whether polyarteritis nodosa and hypersensitivity angitis are entirely separate disorders (14 17 18). However in the typical case polyarteritis nodosa affects large and medium sized arteries and healed and recent vascular lesions are usually found in the same case (12 17 23). Hypersensitivity angitis was described by Pearl Zeek (12 23) as a fulminating disease with a necrotizing vasculitis of small visceral and other vessels particularly those of the lungs. Allergic or hypersensitive mechanisms have been implied but rarely convincingly proved. Cases with a more protracted course have later been included in this group (17). In contrast to polyarteritis nodosa the vascular lesions of hypersensitivity angitis all show the same stage of development (17). The small vessel disease of the present cases may resemble that of hypersensitivity angitis. However the lack of visceral involvement, the intermittent clinical course and the affection of the temporal arteries are atypical features of this disorder.

The histological changes in the temporal arteries of cases 1 and 3 resemble those seen in the clinical syndrome temporal arteritis with the exception that no multinuclear giant cells were observed. However it is not likely that the giant cells are of specific importance for this disease. The giant cells probably represent some kind of foreign body reaction to damaged elastic fibres (7 10) and it is doubtful whether further pathogenetic significance should be attached to them. Several cases of otherwise typical temporal arteritis have been described without giant cells (7 11 16) and giant cell reaction has occasionally been observed also in polyarteritis nodosa (5 7).

Many cases with active temporal arteritis apparently lack or show only vague local temporal symptoms and may present under the clinical picture of polymyalgia rheumatica (1 3 4 6 13 15 16 19 22). Some of these cases have shown active vasculitis also in large muscle arteries (8). Thus there may apparently be transitions between the clinical syndromes temporal arteritis and polymyalgia rheumatica.

The clinical picture in our case 3 was fairly typical of a temporal arteritis. Case 1 had only

vague local temporal symptoms in spite of an active arteritis and the clinical picture resembled that of polymyalgia rheumatica for several months before the parestheses appeared. Case 2 which had no temporal arteritis but a vasculitis of the small vessels surrounding the artery also had many clinical features in common with polymyalgia rheumatica. The central scotoma in this case suggests that the vessels of the eye or optic nerve were also affected.

Widespread vasculitis of small vessels with affection of peripheral nerves as was seen in our cases has not been described either in temporal arteritis or polymyalgia rheumatica. It should be noted however that the neuropathy was recognized clinically only in one case. There is also a considerable chance that a small muscle biopsy may be negative even in widespread vasculitis. Further detailed clinical and postmortem studies are therefore needed in cases of polymyalgia rheumatica and temporal arteritis to investigate the possible relationship to the type of vasculitis described here.

It is apparent from these considerations that our cases do not fit into previously described groups of vasculitis and exact classification of the cases is at present not possible. Regardless of this it should be emphasized that elderly patients with clinical pictures resembling temporal arteritis or polymyalgia rheumatica may develop signs of neuropathy during the course of the disease which is caused by a vasculitis of the small endoneurial vessels. The vasculitis of these cases may remain localized to muscles and peripheral nerves and leave the visceral organs intact.

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LOW MOLECULAR DEXTRAN IN CHRONIC CIRCULATORY FAILURE

Effect Estimated by Lung Diffusing Capacity

P Splvsteen¹ and Eva Nathan

From the Medical Department F Frederiksberg County Hospital Hellerød Denmark

Abstract By means of the steady state method 11 determinations of the lung diffusing capacity (D_L) were made in ten patients suffering from chronic pulmonary diseases before and after infusion of 500 ml of 10% low molecular weight dextran (Rheomacrodex®).

D_L did not change after the infusion. This challenges the assumption that low molecular weight dextran exerts a dilating effect on the lung capillaries and it also indicates either that no aggregation of erythrocytes occurs or that any aggregation is not inhibited by low molecular dextran.

On the basis of the present studies and of those by other authors the question is discussed as to whether low molecular weight dextran might be supposed to improve the supply of oxygen to tissues with threatening gangraena caused by arteriosclerosis better than do other plasma expanders. This does not appear to be immediately probable.

It is emphasized that the investigations do not give any information as to the effect of low molecular weight dextran in acute circulatory disorders such as shock, severe trauma, myocardial infarction etc. in which cases a pronounced aggregation of erythrocytes *inter alia* might exert an essential influence on the course of the disease.

During recent years low molecular weight dextran (Rheomacrodex®) has been used in the treatment of arteriosclerotic vascular diseases with threatening gangraena (4, 8, 10). It was supposed first that by reducing the viscosity of the blood low molecular weight dextran would increase the blood circulation in the hazardous region and secondly that it prevented aggregation of the erythrocytes whereby the surface was increased through which oxygen could diffuse from the blood to the tissues.

A method to show whether an improved supply of oxygen to such small areas of tissue is actually obtained is difficult to establish. How

ever if such an effect is present in the peripheral vessels a similar effect resulting in an increased oxygen uptake must be expected to occur in the lung vessels not least in patients with chronic lung diseases in whom pulmonary circulation is already poor.

This is of interest firstly because it is possible to make determination covering a fairly large capillary network in the pulmonary circulation which may contribute to clarifying the effect on the peripheral circulation and secondly because such an effect might be of practical value in the treatment of patients with chronic lung diseases.

As will be discussed later all possible effects of low molecular weight dextran will tend to increase the lung diffusing capacity (D_L). By measuring D_L it is therefore possible to examine whether low molecular weight dextran has the above mentioned effects on the pulmonary circulation.

The object of the present study was to investigate whether infusions of low molecular weight dextran change the conditions of circulation in the lungs in patients suffering from chronic pulmonary diseases and on the basis of the results obtained to discuss the effect of low molecular weight dextran in patients with peripheral vascular disorders.

METHODS

By the lung diffusing capacity for carbon monoxide is meant the volume of carbon monoxide (in ml) which is taken up per minute over the entire lung surface when the difference between the carbon monoxide tension in the alveoles and the blood is 1 mm Hg.

It was determined by employing the method of Bates et al. (7) slightly modified. Instead of applying a bag-in-the-box principle and determining the respiratory

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Table I Data of subjects and lung diffusing capacity before (D_{L-1}) and after (D_{L-2}) low molecular dextran

Subject	Sex	Age	Diagnosis	D_{L-1} (ml min ⁻¹ mm Hg ⁻¹)	D_{L-2}
C J	♂	59	B E	94	88
H S	♂	75	B	94	82
—	—	—	—	79	85
H H	♂	80	B	85	89
A M	♀	69	A B E C	48	45
A K	♂	64	B A C	113	110
A S	♂	56	B	95	99
K B	♂	52	B E	71	73
K L	♂	60	A B	107	96
P L	♂	22	A	121	103
E J	♂	49	A B E	111	100
Mean				93	88

Symbols A = asthma bronchiale E = emphysema pulm
B = bronchitis chronica C = cor pulmonale

volume by means of a spirometer connected to the box the expired air was collected in a rubber bag and at the termination of the experiment the volume in the bag was determined by forcing the air through a gasmeter. The concentration of carbon monoxide in the air mixture expired in the inspired air and in the end expiratory air was measured by means of a Beckman infra red carbon monoxide analyzer.

In connexion with each examination duplicate determinations were carried out. The interval between the two determinations was 10 to 15 minutes.

Eleven determinations were made in a total of ten patients in eight of these cases an intravenous infusion of 500 ml of 10% low molecular weight dextran was given shortly after the initial determination of D_L . In three cases the dextran was not given until the day after the initial determination of D_L . The 500 ml of 10% low molecular weight dextran were infused over a period of 1 1/2 to 2 hours. The determination of the D_L was made between 30 minutes and 1 1/2 hours after the infusion.

Hence a period of not less than 3 hours passed between two duplicate determinations of D_L . By inserting reasonable volumes of carbon monoxide taken up during the experiments and cleared during the interval between these experiments on the basis of Linderholm's equation (6) it can be calculated that the carbon monoxide present in the blood will only cause the D_L determination to be between 2% and 3% lower in the second session than in the first.

RESULTS

Table I presents the results of determinations of D_L before and after infusion of low molecular weight dextran. In addition the age and sex of the patients and the diagnoses are stated.

The table shows that after infusion of low

molecular weight dextran an average fall in D_L of 5% occurred. This decrease is not statistically significant ($p > 0.1$) and furthermore as previously mentioned a fall of 2-3% must be expected when repeated measurements are made on the same day.

Therefore the conclusion from the determinations is that the D_L was not changed after infusion of low molecular weight dextran and more particularly it was not increased.

DISCUSSION

From the alveoles carbon monoxide must diffuse through the alveolar walls, the capillary walls, the blood plasma, the erythrocyte membrane and the erythrocyte plasma whereupon it is bound chemically to the haemoglobin.

Therefore the D_L depends on the nature of the alveolar walls (thickness, content of fibrous tissue and other physicochemical properties), on the number of functioning capillaries in contact with functioning alveoles and on the content of haemoglobin in the lung capillaries (13). Furthermore the D_L is dependent on the total erythrocyte surface in the lung capillaries. On the other hand if no extreme changes occur such as stagnation of the blood so that the carbon monoxide tension of the blood cannot be considered to be zero, the D_L does not depend on the magnitude of the perfusion (11, 12). Hence by determining the D_L information is obtained as to whether a certain plasma expander (in the present case low molecular weight dextran) influences solely the perfusion or whether it has some other of the effects mentioned above.

In small vessels low velocities will produce a tendency to aggregation of the erythrocytes and consequently a tendency to a high viscosity of the blood. From a theoretical point of view this will create a tendency to non uniform distribution of the blood in the capillaries (3) which can be illustrated as follows:

If the blood had to pass through all capillaries the rate of velocity would perhaps be so low and consequently the aggregation of erythrocytes so pronounced that the circulation would stop completely. On the contrary if the blood were to pass through fewer vessels a suitably high rate of velocity without any aggregation of erythrocytes worth mentioning could be maintained. In

the case of an uneven distribution of the perfusion of this nature reduced blood viscosity or increased perfusion in the afferent vessels would either give rise to an increased velocity in the existing vessels or to dilatation of new vessels.

As is the case when other plasma expanders are infused infusion of low molecular weight dextran will increase the filling pressure of the heart and thereby the cardiac output (5). The perfusion through the lungs must therefore be increased. Furthermore low molecular weight dextran is supposed to produce a decrease in the blood viscosity and thereby to contribute to a greater extent to the opening of new vessels.

However the investigations show that the D_L is not increased by infusion of low molecular weight dextran. Therefore the content of erythrocytes in the lung capillaries cannot be increased and this opposes the assumption that the lung capillaries are considerably dilated.

The absence of an increase in D_L also indicates either that no important aggregation of erythrocytes is present in the pulmonary circulation or that any existing aggregation is not counteracted by administration of low molecular weight dextran. Disaggregation of the erythrocytes per se would enlarge the surface of the erythrocytes and thereby increase the D_L .

Hence the effect of an infusion of low molecular weight dextran in patients suffering from chronic pulmonary diseases does not seem to differ much from that observed in healthy persons after infusions of albumin solutions. Also in these cases an increase in cardiac output is seen not associated with an increase in the D_L (12).

The results of the present investigations are in accord with those reported by Korsgren (5) obtained from studies of healthy persons and patients suffering from mitral disorders. His examinations showed that infusions of low molecular weight dextran did not impair the pulmonary resistance or increase the blood volume of the lungs. However the blood volume measured by applying the Korsgren method comprised a considerably greater volume than that of the pulmonary capillaries including the entire volume of blood found between the pulmonary artery and the left atrium. Thus when the Korsgren method is employed the possibility cannot be discounted that the blood is displaced from one part of the lung vessels to another during the infusion. Since

the infusion resulted in an increase in pressure in the pulmonary artery it might be supposed that the blood was displaced from the arteries to the capillaries or the veins. The investigations reported in the present paper show that the D_L does not increase. Therefore any displacement of the blood cannot be to the capillaries.

Consideration must be given as to whether the absence of an increase in the D_L subsequent to infusion of low molecular weight dextran might be caused by the fact that prior to the infusion the dilatation of all the capillaries was maximum. However in patients suffering from chronic bronchitis and emphysema the D_L increases during exercise (1, 7, 9). This increase is more pronounced than that which might be caused solely by an increase in ventilation (1) and this indicates that it is caused by an increase in the capillary blood volume in the lungs. Korsgren (5) found that an infusion of low molecular weight dextran succeeded by infusion of acetylcholine increased the blood volume of the lungs although low molecular weight dextran alone did not exert this effect. Therefore it does not appear probable that the capillaries were incapable of dilating.

Hence the results do not give any reason for supposing that infusion of low molecular weight dextran increases the surface through which carbon monoxide (and oxygen) diffuses or that it has any effects on the pulmonary circulation in patients suffering from chronic lung disorders other than those exerted by other plasma expanders.

As regards the peripheral circulation Korsgren showed also that the resistance is reduced after infusion of low molecular weight dextran or other plasma expanders. Hence in the peripheral circulation plasma expanders must cause a vaso-dilatation but it is uncertain in which part of the circulation such dilatation occurs. As previously mentioned in order that low molecular weight dextran may produce an increase in the diffusion surface a disaggregation of the erythrocytes must be obtained resulting in an increase in the erythrocyte surface and possibly also in a dilatation of the capillaries. Although the mechanisms governing the regulation of the peripheral and the pulmonary circulation doubtless differ widely it appears most unlikely that low molecular weight dextran as far as disaggregation of the erythrocytes is concerned should have dif-

ferent effects in the peripheral and the pulmonary circulations. To this should be added that in tissues with incipient ischaemia the local hypoxia in itself presumably has produced maximum dilatation of the capillaries before the infusion is started.

Consequently the results of the investigations do not provide any basis for supposing that infusion of low molecular dextran will result in a better supply of oxygen to peripheral tissues e.g. to tissues with threatening gangraena because of arteriosclerosis than in the case when other plasma expanders are infused.

It should be emphasized that the investigations do not give any information as to the effect of low molecular weight dextran in acute circulatory disorders such as shock, severe traumatic tissue injuries, myocardial infarctions etc. in which cases a pronounced aggregation of erythrocytes inter alia might exert an essential influence on the course of the disease.

ACKNOWLEDGEMENTS

The study was supported by grants from the Danish State Research Foundation and Miss P. A. Brandts Foundation.

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DIABETIC COMA WITHOUT KETOACIDOSIS

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Abstract As early as the 19th century diabetic coma without ketoacidosis (d.c.w.k.) was considered by some authors to be a disease differing from the common diabetic coma. During recent years more than 60 cases have been published in the literature still carrying a mortality of 50%.

We have collected another five cases. None of the patients was in deep coma and only one died. Four of the patients had previously been treated with thiazides which in some of the cases might have induced the diabetes.

The mechanism of the development and the treatment of d.c.w.k. is discussed. Administration of insulin and large quantities of fluid parenterally is necessary if the high mortality is to be reduced. For rehydration purposes we prefer hypotonic saline containing 100 mEq NaCl per litre.

In a paper concerning diabetic coma associated with renal disease Warburg (26) in 1925 presented a survey of the history of the diabetic coma. It appears from this survey that in the middle of the 19th century it was supposed that the deterioration of consciousness in comatose patients was the result of acetone intoxication. As early as in the 1880's however it was realized that not all patients suffering from diabetic coma had acetonuria. In addition to the classical type of coma associated with acetonuria and deep breathing described by Kussmaul and others, French (8) and Dreschfeld (7) presented another type characterized by hyperglycaemia and sudden collapse or "cardiac failure" accompanied by weakness, cold extremities, weak pulse, drowsiness and atypical Kussmaul breathing but without acetone in the expired air or the urine. Death occurred within 10 to 20 hours.

Similar cases have been described by Joslin (15) and Rosenbloom (20).

Warburg's material comprised four patients

suffering from diabetic coma without acetonuria or with only slight acetonuria.

These four patients were suffering from renal disease and Warburg was inclined to believe that for this reason the ketones present in the blood could not be excreted with the urine. Consequently Warburg supposed that ketonaemia was present.

In three of the patients this assumption might be correct since in these patients a smell of acetone and increased urinary excretion of ammonia were observed. On the other hand it was doubtful whether the fourth patient had acidosis and ketonaemia. This patient was a 57 year old man who had previously been in good health. He was admitted to hospital deeply comatose, his blood sugar on admission was 670 mg per 100 ml. He was dehydrated occasionally with deep breathing which however was not typical (i.e. of Kussmaul type). The expired air did not smell of acetone, no acetonuria was present and the urinary excretion of ammonia was not increased. The patient was treated with insulin and saline infusions. His condition improved rapidly and he was discharged from hospital initially without insulin but later such treatment had to be instituted.

Thus it has been observed a long time ago that diabetic coma may occur without being associated with acetonuria but the classification of such cases as an independent type of diabetic coma was not maintained. Interest was revived when in 1957 de Graef and Lips (6) and Sament and Schwartz (23) published two cases of diabetic coma without ketoacidosis (d.c.w.k.). Since that time more than 60 cases have been described (14, 18). The condition is still considered to be rare although the diagnosis is made with increasing frequency and the disease seems to be more

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common than formerly supposed. The lethality is high. This is apparent from a survey of the literature over the last decade which shows that 28 out of 53 patients have died (18).

During the last four years five patients have been admitted to the medical departments B and F of the Frederiksborg County Hospital under the diagnosis of d.c.w.k. However it should be stated that these patients were rather precomatose, than actually comatose. The case reports of these patients are given below with the object of drawing attention to the frequency and serious prognosis of this entity and to the varying concepts in respect to the pathogenesis and treatment. Three of these cases have been reported previously by Grønbæk and Kjeldsen (10) with the object of illustrating the diabetogenic effect of thiazides.

CASE REPORTS

1 Case B 554/63-64

A 69-year-old extremely obese female. One of her cousins had diabetes mellitus. For a period of four years the patient had been treated with chlorothiazide because of heart failure and during the last five weeks she had also received digitalis.

For a couple of weeks the patient had vomited and had diarrhoea and after that time she had complained of increasing thirst and polyuria. She was admitted on April 25, 1963. On admission she was soporous and dehydrated. Her blood sugar was 932 mg per 100 ml. No acetonuria or polyphagia was present. Standard bicarbonate was 29.5 mEq/l, serum creatinine 2.2 mg/100 ml. ECG revealed left axis deviation and Cohen effect.

During the first 24 hours the patient was given 180 IU of insulin and 4300 ml of fluid half of which was given parenterally as a hypotonic electrolyte solution (see later). Subsequently the blood sugar was 273 mg/100 ml and the patient was completely alert. The serum creatinine became normal within 14 days. Since that time the patient's diabetes has been stabilized on diet alone although treatment with chlorothiazide and digitalis was still required because of the cardiac insufficiency.

2 Case F 240/60-61

A 77-year-old moderately obese female. No family history of diabetes mellitus. Treated over a period of two years with thiazide and digitalis because of cardiac failure. Furthermore the patient received chlorpromazine and used considerable amounts of analgetics.

After three weeks increasing thirst, polyuria, fatigue and vomiting, the patient was admitted to hospital on December 14, 1963. She was slightly dehydrated and drowsy. The blood-sugar was stated to be higher than 387 mg/100 ml and two hours after administration of 40 IU of insulin it was 726 mg/100 ml. No acetonuria was present. The standard bicarbonate was 16.4 mEq/l and serum creatinine 3.6 mg/100 ml.

During the first 24 hours the patient received 178 IU of insulin and 2000 ml of isotonic electrolyte solution parenterally and also large amounts of fluid orally. Subsequently the blood sugar fell to 296 mg/100 ml.

On discharge the patient still received 20 IU of insulin daily. The diuretics had been withdrawn.

It should be stated that as early as 1961 slight renal failure associated with a serum creatinine value of 1.6 mg/100 ml had been observed. Furthermore at subsequent follow-up examinations the serum creatinine was found to be increased (up to 6.0 mg/100 ml) and the patient had often had low standard bicarbonate (as low as 12 mEq/l) without any acetonuria.

3 Case F 761/64-65

A 59-year-old very obese female. No family history of diabetes mellitus. In 1942 the patient underwent strumectomy because of exophthalmic goitre. For a period of about two years the patient had had a tendency to oedema and slight hypertension for which she received chlorothiazide. Furthermore she was treated with the ethylperazam maleate (Torecan®), phenylbutazone (Butazolidin®), 700 mg daily chlorprothixene (Truxal®), 45 mg daily reserpamine (Rescamin®), 1.5 mg daily.

During the previous month the patient had complained of thirst, loss in weight and impairment of vision. Furthermore she had had loose stools for the previous two weeks.

She was admitted to the hospital on August 8, 1964. During the preceding 24 hours she had had nausea and vomiting and had been drowsy. The temperature was 39°C, the blood-sugar 1.18 mg/100 ml. There was no acetone in the urine. The standard bicarbonate was 23.3 mEq/l. At the clinical examination the patient was dehydrated. The serum creatinine was 3.3 mg/100 ml and the serum protein 9.4 g/100 ml.

During the first 24 hours 440 IU of insulin and 4850 ml of fluid were given mainly as infusions of hypotonic electrolyte solutions. The blood sugar was reduced to 570 mg per 100 ml. The patient was then completely alert, the temperature normal but anuria was present. During the following 24 hours the patient received 304 IU of insulin and 4750 ml of fluid whereupon the diuresis rose to normal values. The blood sugar and serum creatinine became normal.

On discharge the patient's diabetes was treated with diet. Insulin was not required. The blood pressure ranged around 200/100 mm Hg. It was not found necessary to continue the chlorothiazide treatment.

4 Case F 621/64-65

A 54-year-old slightly obese female. No known family history of diabetes mellitus. The patient had suffered from diabetes mellitus for ten years. For some time the patient had been treated with diet and oral hypoglycaemic agents but later she stopped taking the drug and it was doubtful whether she has kept her dietary schedule.

For many years the patient had suffered from gastric dyspepsia. The symptoms became more pronounced and on August 13, 1964 she was admitted to hospital because of vomiting, acid aqueous vomiting and increasing drowsiness. On admission she was extremely de

hydrated. The blood sugar was 881 mg/100 ml there was no acetone in the urine. The standard bicarbonate was 41.9 mEq/l, serum creatinine 9.1 mg/100 ml, serum potassium 2.9 mEq/l, serum chlorides 47.2 mEq/l and serum sodium 140 mEq/l.

During the first 12 hours the patient received 250 IU of insulin and 6850 ml of fluid first as infusions of hypotonic solutions later—when the above electrolyte analyses were available—as infusions of potassium and sodium chloride. Because of falling blood pressure serum and blood were ill given.

The blood sugar fell to 99 mg per 100 ml and the patient regained complete consciousness. During the night she suddenly aspirated large volumes of stomach contents. The patient died in spite of tracheo-bronchial suction, respirator treatment, blood transfusions and subsequent cooling because of severe hyperthermia.

Autopsy revealed severe pulmonary oedema, a large fistula between the gall bladder and the duodenum and slight gastritis.

5 Case F 1051/66-67

A 81 year-old slightly obese female. No known family history of diabetes mellitus. Over a period of several years the patient had been treated with digitals and a thiazide diuretic because of cardiac failure. Furthermore she was given tolbutamide (Rastamon®) 500+250 mg daily because of slight diabetes.

She was admitted to hospital on August 3 1966 after having been dizzy, drowsy and feverish for a few days. The temperature was 40°C, the blood-sugar 607 mg/100 ml. There was no acetone in the urine. The standard bicarbonate was 23.8 mEq/l, the serum creatinine 3.1 mg/100 ml. During the first 24 hours the patient was given 240 IU of insulin and 7100 ml of fluid mainly as infusions of hypotonic electrolyte solutions.

The next day the blood sugar was 218 mg/100 ml. During the following 4 hours another 168 IU of insulin and 4200 ml of fluid were given orally and parenterally. Although no focus of infection had been found the patient was treated with penicillin. The temperature became normal two days after admission.

Later the patient developed severe urinary infection and urinary retention, and an indwelling catheter was introduced. In addition a large bed-sore developed in the sacral region.

The patient was transferred to a residential home where she died two months later. She was treated throughout with insulin Retard® 12 IU a.m. and 8 IU p.m.

DISCUSSION

The five cases of d.c.w.k. reported were all seen within a period of four years. During the same period twenty five patients suffering from classical diabetic coma or precoma associated with ketoacidosis had been admitted. The non ketotic coma consequently does not appear to be extremely rare as previously supposed.

Various hypotheses have been advanced as to

why some patients with diabetic coma do not develop ketoacidosis.

Many of the cases described in the literature occurred in connexion with the administration of thiazides. Therefore Schwab et al. (24) supposed that the mechanism might be that the thiazides gave rise to alkalosis. The latter would then neutralize the metabolic acidosis caused by the diabetes.

This hypothesis does not appear very justifiable. Firstly the thiazides produce a slight alkalosis only which can hardly neutralize a severe diabetic acidosis. Secondly a neutralization per se of the acidosis would not be able to remove the ketones from the blood and therefore could not prevent the appearance of acetonuria.

Another hypothesis advanced by Antoniadou (1) is based on the fact that the disease occurs in patients with maturity onset diabetes. Such patients are known to be able to produce insulin. (4) Antoniadou believes that in these patients the insulin in plasma is bound to protein which would reduce the influence of insulin on muscle tissue and consequently the uptake of glucose in such tissue. On the other hand in the fat tissue a factor is assumed to be present which is able to split the insulin from the protein so that the influence of insulin in fat tissue is maintained. This results in inhibition of lipolysis and therefore also of the formation of ketone bodies. In this way this hypothesis could explain the occurrence of hyperglycaemia without ketosis. However the hypothesis is purely speculative based on experiments *in vitro* on animal tissue. From experiments *in vivo* it was not possible to demonstrate any insulin effect on fat tissue of plasma from a patient with d.c.w.k. (14).

Up to the present the hypothesis advocated by Benedetto et al. (2) is the most plausible. These authors collected 27 cases of d.c.w.k. from the literature and demonstrated firstly that the disorder is most common among elderly obese patients and secondly that prior to the development of coma many of the patients had consumed large quantities of carbohydrates. Since in such patients administration of carbohydrates will cause an increase in the serum insulin (4, 19) the initial content of insulin in the tissues will be high and this prevents the development of ketoacidosis. Furthermore the insulin reserve in these patients is reduced (19) and consequently continued ad-

ministration of carbohydrates must be supposed to exhaust the beta cells so that the production of insulin is reduced without necessarily being stopped entirely. This intensifies the hyperglycaemia. Hyperglycaemia *per se* inhibits the formation of ketone bodies (16). The development of ketoacidosis during this stage is thus counteracted by the small amount of insulin present in combination with the pronounced hyperglycaemia.

From this hypothesis it will be possible also to explain why d.c.w.k. is a frequent occurrence in patients treated with thiazides. As the result of experiments both *in vitro* (9) and *in vivo* (21) it has been shown that thiazides inhibit the secretion of insulin from the beta cells. Therefore it is reasonable to suppose that in diabetics treated with thiazides even relatively small amounts of carbohydrates are able to exhaust the beta cells and thereby to give rise to d.c.w.k.

The mechanism described may explain the development of d.c.w.k. in all the patients comprised in our studies. In two of the patients diabetes mellitus had been diagnosed previously. One of these patients disregarded the dietary regulations completely while the other had received a thiazide compound. Diabetes mellitus had not been diagnosed previously in the remaining three patients. Therefore they had not been on diet and in addition had been treated with thiazides.

Treatment must be directed against the hyperglycaemia and the dehydration. To achieve this intensive fluid therapy and often high doses of insulin are needed (27). Whereas the insulin treatment has not given rise to any major problems, opinions differ as to which fluids should be used for infusion. Sament (22) and Haapanen (11) think that isotonic sodium chloride solution should be employed also in cases when the serum sodium is increased. Their reason is that apart from the fluid deficiency there is also a genuine lack of salt which is only masked by the pronounced dehydration.

Other authors (12, 13) are opposed to treatment with isotonic sodium lactate, sodium chloride and sodium bicarbonate as they claim that these fluids will cause an increase in a possibly existing hypernatremia and be unable to correct the hyperosmolality. Hence Halmos et al. (12) suppose that the fatal outcome in two of their four cases might have been avoided if instead of saline and sodium lactate the patients had been

given isotonic glucose which in their opinion is the correct fluid to be used for infusion. Nelson (17) elaborates further on this assumption emphasizing that first and foremost these patients require water. When given insulin intravenously together with the glucose solution the glucose will be assimilated almost as quickly as it is infused and water will become available.

Because patients with d.c.w.k. suffer from pronounced water deficiency and since possibly there is a genuine but relatively smaller salt deficiency it appears most rational to treat with hypotonic electrolyte solutions as recommended by e.g. Christiansen et al. (5) and Bradley (3). Sjöberg (25) who also used hypotonic saline suspected that this solution gave rise to haemolysis in one of his three patients but he does not state the quantities and concentrations of the fluid or the infusion rate.

Initially our patients were treated in the same way as our comatose patients with ketoacidosis. The three most seriously ill patients were given a hypotonic solution (composition (mEq/l): Na^+ 100, Cl^- 70, lactate $^-$ 30) and the other two were treated with an isotonic solution which was hypotonic in respect to electrolytes (composition (mEq/l): Na^+ 45, K^+ 25, Mg^{++} 5, Cl^- 45, H_2PO_4^- 10, acetate $^-$ 20, sorbitol 50). The content of lactate and acetate respectively in these infusion solutions is justified in patients with ketoacidosis but not in patients without ketoacidosis. In patients without ketoacidosis we consider it more appropriate to use a hypotonic sodium chloride solution e.g. containing 100 mEq Na^+ and 100 mEq Cl^- per litre. In other diseases we have extensively used solutions in similar concentrations without encountering complications; therefore the risk of haemolysis cannot be great.

Even if the mortality observed in our material is considerably lower than average with only one death among the five patients, no conclusions can be drawn as to the efficiency of the treatment on the basis of the results obtained, firstly because the material is very limited and secondly because all the cases were mild.

The personal experience gained by other authors is also very limited and their principles of treatment are based more on theoretical considerations than on experience.

Therefore it is important that this condition is recognized, the diagnosis established early, proper

treatment instituted and the result published in order that an efficient and well founded therapy may be developed and the high death rate reduced

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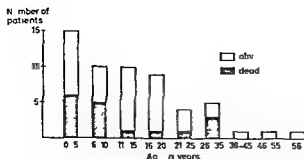


Fig 1 Total material Age distribution related to mortality

tion Out of the 56 cases included in the following presentation, 19 were females and 37 males. The age at operation is shown in Fig 1. Three adult patients had a history of subacute bacterial endocarditis before the total correction.

PRE-OPERATIVE FINDINGS

Twenty-one patients were grouped in function class II, nineteen in class III, and sixteen patients in class IV. No patient belonged to function class I.

The localization of the systolic murmur was determined and its intensity graded according to Levine and Harvey (17). The maximum intensity was as a rule noted at the left sternal border in the third to fifth intercostal space. Table I shows the character of the murmur in 55 of the 56 patients. In the youngest patient, fifteen months at operation, no murmur could be heard. The systolic murmur was of grade 3 or 4 in 46 cases and pansystolic in 31.

The hematocrit reading was 45% or less in six patients, 46 to 55% in 22 and between 76 and 85% in six.

Table I The systolic murmur in 55 cases of Fallot's anomaly prior to total correction

Grade	Early systolic	Midsystolic	Late systolic	Spindle shaped	Pansystolic max			Total
					Early systolic	Midsystolic	Late systolic	
II	4	0	0	1	0	0	0	5
III	7	2	0	5	0	5	2	27
IV	1	1	0	2	3	9	2	18
V	0	0	0	0	1	2	1	4
VI	0	0	0	0	1	0	0	1
Total	12	3	0	8	11	16	5	55

Table II The systolic pressure gradient RV PA (mm Hg) in 36 cases of Fallot's anomaly

Systolic pressure gradient	No of pat	Systolic pressure gradient	No of pat
RV PA			
60-70	8	111-120	5
71-80	8	121-130	1
81-90	9	131-140	1
91-100	0	141-150	2
101-110	2	Total	36

Thrombocytes less than $100\,000/\text{mm}^3$ were noted in six cases. Three of these had a thrombocyte count less than 40. In two cases the serum bilirubin was elevated and two had pathological bromsulphalein tolerance tests. In all six cases a severe cyanosis was present and the hematocrit reading was 70% or more.

Definite electrocardiographic signs of right ventricular hypertrophy occurred in 41 cases and were suspected in 14 other cases. Hypertrophy of the right atrium was electrocardiographically evident in 21 cases.

The relative heart volume was normal or slightly elevated in most cases. The four oldest patients had a more pronounced enlargement of the heart.

At right heart catheterization it was possible to pass to the pulmonary artery in 36 out of 56 cases. The peak systolic gradient between the pulmonary artery and the right ventricle is shown in Table II. In 25 cases the gradient was between 60 and 90 mm Hg and in 11 cases more than 100 mm Hg.

With the aid of data from the heart catheterization, the angiocardiology and the findings at operation, the pulmonary stenosis could be evaluated. One patient had an isolated valvular stenosis. 21 patients had isolated infundibular stenosis. In 34 cases the combination of valvular and infundibular stenosis was present.

Fifteen patients had supraventricular malformations of the pulmonary artery as well. In eight cases hypoplasia of the main stem was present and three of these had hypoplasia or aplasia of the left branch as well. Two cases had hypoplasia of the left branch and three cases had malformation of only the right branch. In two patients finally

Table III Vascular congenital malformations in 56 cases of Fallot's anomaly

Atrial septal defect	2
Fistula from left coronary artery to bronchial artery	3
Duplication of right coronary artery	1
Aplasia of the pulmonary valves	2
Bicuspidal pulmonary valves	1
Bicuspidal aortic valves	1
Anomalous pulmonary vein to superior vena cava	1
Left sided superior vena cava	5
Situs inversus	1

both pulmonary branches were malformed but not the main stem

Table III shows other malformations of the cardiovascular system. Out of the two patients with atrial septal defect one had bicuspid aortic valves and one had persistent left superior vena cava

PER OPERATIVE AND IMMEDIATE POST OPERATIVE FINDINGS

Fig 2 presents the number of patients operated on each year and the mortality year by year. Seventeen patients died after operation: three of them immediately after the total correction and the other 14 within three and a half months after the operation. Of the 17 cases, ten were women and seven men.

In the sequel, the factors which might be of importance for the operative results will be commented on. These factors are chiefly the age at total correction, the severity of the pulmonary stenosis, the type of complicating cardiovascular anomalies, the preceding palliative operation, and finally the technique at operation including the length of the extra corporeal circulation and the primary result with regard to the pressure in the right ventricle.

The median age of the 17 patients dying after operation was 7 years (range 1-34 years) compared to the median age of 11.5 (range 1-56 years) of the whole material. The median age for the surviving cases was 12 years (range 2-56 years). From Fig 1 it is clear that the age group of 10 years or less at operation represents one third of the material and two thirds of the mortality.

Palliative treatment had been performed in 16 cases (Fig 2). Twelve of these were operated on

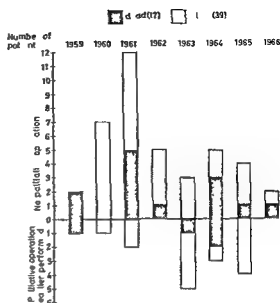


Fig 2 Fifty six cases of Fallot's anomaly. Total correction performed in 1959-1966.

by the Blalock-Taussig procedure and in four cases the shunt was non-functioning at the time of total correction. In three cases the procedure of Brock had been used, and in one case an aortico-pulmonary window according to Potts had been made. Of the 16 cases treated palliatively, four died at the total correction. The corresponding figures for the material not earlier operated on were 40 and 13 respectively.

Fig 3 shows that when the functional capacity is low, the mortality increases. The hematocrit value could be taken as a sign of the severity of the heart disease. Thus it was found that in the group with the highest hematocrit readings, six patients with values between 76 and 85, four patients died, and in the group of six patients having hematocrit readings less than 45, only one died. In the group of 22 patients with hematocrits between 46 and 55, seven died at or after the total correction.

During the period 1959-1961, 25 patients were operated on. Ten of these cases (40%) had total AV block at the correction; in five cases the block was not permanent. During the period 1962-1966, 31 patients were operated on and complete AV block occurred in four cases (13%). In the group of surviving patients for the whole period 1959-1966, total block occurred in seven (18%).

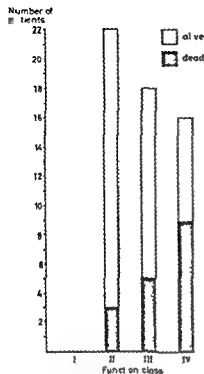


Fig 3 Mortality related to function class in 56 cases totally corrected for Fallot's anomaly

and in the group dying at or after operation complete AV block occurred in seven (41%).

At the registration of the pressure in the right ventricle immediately after the correction the pressure was found to be reduced to 50% or more in 26 of the 39 surviving cases (70%) as compared to 5 of 17 (30%) in the group of patients dying at or after operation. Two of the 11 patients with a systolic pressure gradient more than 100 mm Hg pre-operatively died at the total correction.

The perfusion time during the extra-corporeal circulation was 82.6 min (49–124 min) in the group of survivals and 86.3 min (47–134 min) in those who died.

The main cause of death was septicemia in two cases in two other cases brain damage probably caused by the deep hypothermia was judged to be the cause of death.

Of the 34 patients with a combination of valvular and infundibular stenosis ten died. Of the 21 patients with an isolated infundibular stenosis seven died. Eight of 15 patients with supraventricular changes of the pulmonary artery died at the operation. In the group of 15 patients with other

vascular malformations eight died at operation. Six patients had both supraventricular pulmonary malformations and other vascular anomalies four of these patients died.

FOLLOW UP EXAMINATION

Thirty six of the surviving 39 patients were investigated post operatively after between 5 and 82 months (medium 31 months). In three cases it was not possible to perform a complete post operative examination.

The hematocrit value was normalized in all cases except in the 43 year old man whose value still was 53%. The explanation of his high hematocrit value is probably that he has a cystic renal disease. His nonprotein nitrogen value was 55 mg%. No shunt could be demonstrated at post operative cardiac catheterization or cardioangiography. The arterial oxygen saturation was over 90%.

In the six cases with decreased thrombocyte values and also signs of increased bleeding tendency the thrombocytes increased to normal during the immediate post operative period.

One case had a complete block post operatively and an atrial triggered pacemaker was implanted with good result. Before the implantation the heart volume was 800 ml/m² of body surface and after implantation normal. Another case was re-operated because of aneurysm of the outflow tract the outflow prosthesis of pericardium was changed to a prosthesis of teflon instead. At re-operation a small residual ventricular septal defect was noted the defect was resutured and the post operative course was uneventful. The oldest case a 56 year old man was found to have a residual left to right shunt of 3 l/min at rest. The arterial oxygen saturation was 98% at rest and 96% at work. Angiocardiology from the left ventricle showed a 3–4 mm wide communication from the right sinus Valsalvae to the outflow tract of the right ventricle. No ventricular septal defect could be demonstrated. Having regard to the age of the patient and the relatively good post-operative condition no new operation has been performed on this patient. At the autopsy of a 4 year-old boy dying three months after total correction a small ventricular septal defect was found. This patient who had signs of extra pyramidal lesions post operatively was found to have a diffuse brain

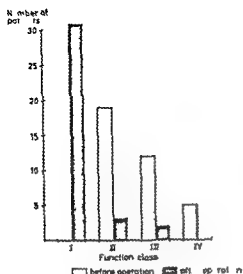


Fig 4 Function class before and after total correction in 36 cases of Fallot's anomaly

lesion with destruction of the ganglionic cells especially in the pallidum as found in hypoxia

When grouping the patients in function classes 31 of 36 were found to be in class I post-operatively. Twenty patients changed from class II to I, nine from class III to I and two from class IV to I. The two cases having function class III post

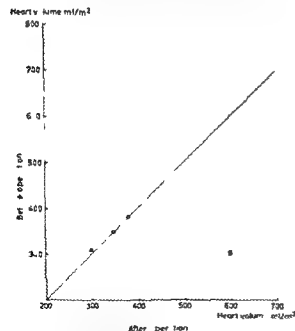


Fig 5 Heart volume before and after total correction in 34 cases of Fallot's anomaly

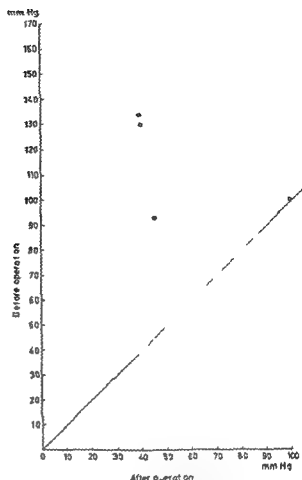


Fig 6 The right ventricular systolic pressure at rest in 30 cases of Fallot's anomaly before and after total correction

operatively were the two oldest 55 and 56 years of age at the time of operation. None of the 36 cases had deteriorated (Fig 4).

A complete right bundle branch block was noted in all cases except in a 22-year-old man who still had sinus rhythm post-operatively. In another patient bundle branch block was not present immediately after operation but was noted six months after operation and onwards.

The relative heart volume had increased moderately in 19 patients, was unchanged in ten and had moderately decreased in five (Fig 5).

At the post-operative cardiac catheterization an overall good reduction of the right ventricular pressure at rest was noted (Fig 6). A 3-year-old girl showed a pressure of about 100 mm Hg both

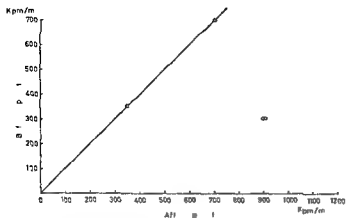


Fig 7 Working capacity in 18 cases of Fallots anomaly before and after total correction

pre and post operatively. She was in function class II before and class I after operation.

An improvement of the physical working capacity was obtained in most of the 18 patients whose working capacity could be compared before and after operation (Fig 7).

DISCUSSION

The mortality at total correction of Fallots anomaly varies in different materials. Kirklin et al (14) had a mortality of 17% in a group of 110 patients operated on during the period 1958–1959. If only the cyanotic cases were included the mortality in this material was found to be 23%. Bahnson et al (1) report a material of 147 cases with total correction performed between 1956 and 1961. In the cyanotic group the mortality was 32% and in the non cyanotic 22%. In a Swedish material including 94 cases operated on from 1956 to 1962 Ekestrom (8) noted a mortality of 43%. Zerbini et al (25) had a mortality of 31% during the period 1958–1961 but only 13.5% for the period 1962–1964. Neutze (21) reported a mortality of 17% in one hundred patients totally corrected 1960–1964. Barnard and Schrire (2) finally had a mortality of only 12% in a material including one hundred patients corrected 1961–1965.

The mortality of 30% in our material is in rough accordance with earlier reports in which increased mortality was found in groups with severe disease and the highest mortality in the group with the most severe cyanosis. The finding of the lowest mortality in the age group 11–25 years is in accordance with earlier reports. This group which consisted of 23 patients in the

present material had a mortality of only three cases.

Two main causes of death can be seen: surgical complications and factors connected with the nature of the material.

The two cases of septicemia and the two cases of brain damage after deep hypothermia belong to the first group. Deep hypothermia is not used any longer. In one case in which a big coronary artery was severed the death was due to right ventricular failure. In several other cases in which such an evident local cause could not be found right heart failure was probably a main factor contributing to the fatal outcome. The type of incision in the heart is of interest not only from the point of view of surgical technique but also from the hemodynamic aspect. There is no doubt that the longitudinal incision in the right ventricle originally used makes the operation technically easier. From the aspect of the arrangement of the muscle fibers and the coronary vessels a transverse incision seems to be less harmful and has been said to involve less risk for right heart failure (10). In the present material a longitudinal incision was used in the first part of the series and later a transverse incision was performed or even an incision from the right atrium. In this small material it is difficult to estimate the importance of the type of incision but a certain trend in favor of the transversal incision can be traced. In the group of patients surviving a longitudinal incision had been made in three of five cases and a transversal or a right atrial incision in two of five. The corresponding figure for the mortality group was four of five and one of five respectively.

In the present series anoxic arrest with moderate hypothermia and also in some cases local hypothermia of the heart was used. It would possibly have been preferable to use shorter periods of arrest not exceeding five minutes of the coronary artery flow combined with moderate hypothermia though this factor can hardly be of major importance. An argument in favor of intermittent shorter periods of arrest is that the risk of surgical heart block will be reduced when operation is performed with beating heart allowing continuous control of the ECG. The occurrence of complete AV block is always a latent risk at operation a risk that should be reduced with increased technical experience which could also be noted in our series. Even if it is possible to treat the block with pacemaker it is a serious complication which has probably contributed to the mortality. In the group of surviving patients a complete block was found in seven cases (18) and in the group dying after operation it was also found in seven cases (41).

For obvious reasons the pressure measurements made at operation have limited significance. A reduction of the right ventricular pressure to 50 or more of the systemic pressure was however twice as usual in the group of survivals as in the mortality group. The perfusion time was not significantly different in the two groups. Probably the duration of the perfusion indicates the severity from a technical point of view and should perhaps not be of prognostic significance.

The other main factor in the analysis of the mortality was the characteristics of the individual cases. As mentioned above the greatest mortality was noted in the youngest patients among whom five of eleven operated on before the age of five years died. In these cases it should thus be advisable to give a palliative treatment usually an anastomosis of the Blalock-Taussig type as a first surgical procedure. This has also been our policy during the last years. Left heart failure was estimated to be a contributing cause of death in at least three cases. It is possible that this could have been avoided by an earlier palliative treatment. In the surviving group palliative treatment had been performed in one third of the cases and in the mortality group in one sixth. Thus an earlier palliative treatment did not seem to involve an increased operative mortality.

The greatest risk in primary total correction is

then noted in the youngest children with severe stenosis in whom also an outflow prosthesis was necessary. Wolf et al. (24) among others have reported the greater mortality in cases with outflow prosthesis. The same tendency could possibly be traced in our material in which seven of the 22 cases with outflow prosthesis died.

The three oldest patients 43, 55 and 56 years old at operation all survived. None of them had earlier been treated with palliative surgery. Cooley et al. (4) reported seven cases totally corrected of whom all were more than 35 years old. Two of these died at operation and they were cyanotic. Friesinger and Bahnson (9) report a successful total correction in a 54-year-old man. Probably an increased cyanosis as a sign of the severity of the pulmonary stenosis will increase the operative risk in the higher age groups. It is possible that the oldest patients with the anomaly of Fallot have less severe pulmonary stenosis in earlier years and the gradual increase of the stenosis could then be explained by an increase of the muscular hypertrophy of the outflow tract. These cases are not cyanotic from the beginning but turn out to be cyanotic when the stenosis is so severe that the shunt becomes reversed. A good collateral circulation from the bronchial arteries has also been ascribed as a prerequisite for lengthy survival in Fallot's anomaly.

In eleven patients a systolic pressure gradient over the pulmonary valve of more than 100 mm Hg was noted; two of these cases died at the operation. As the number of cases in which the gradient measured pre-operatively is limited it is difficult to say whether a large gradient and high mortality are correlated. In contrast to the finding of Wolf et al. (24) we could not find any definite increased mortality in patients with both valvular and infundibular stenosis. It is also clear that the mortality increases if there are supravalvular changes of the pulmonary artery and also other vascular malformations.

The patients surviving total correction show a definite improvement which could also be demonstrated objectively. The hematocrit reading was normalized except in one case with a cystic congenital renal disease. The low thrombocyte values in six patients became normalized after operation. Somerville et al. (22) investigated 50 cases with congenital heart disease with 40 cyanotic cases in the group. In the cyanotic group

a tendency to thrombocytopenia and deficiency of fibrinogen was noted. The most frequently encountered abnormality was depression of thromboplastin generation. The degree of impairment of thromboplastin generation was greatest in the patients with the greatest increase in hemoglobin. No abnormality was found in the ten patients with acyanotic congenital heart disease. Kornhuber and Gutheil (16) are of the opinion that the oxygen consumption of the thrombocytes is high. Hypoxemia as in cyanotic heart disease should therefore give a shorter survival time and in the most hypoxic cases a thrombocytopenia is also noted.

The post-operative right heart catheterization showed a good reduction of the right ventricular pressure in most cases. Yet most cases still had an increased right ventricular pressure which could be explained by the fact that part of the stenosis persisted.

No patient was reoperated because of reappearance of ventricular septal defect. The explanation is probably that most cases were operated on with a prosthesis instead of direct suture. At the reoperation of the 7 year old boy for an aneurysmal dilatation of the outflow prosthesis a minimal defect was found at the place where the ventricular septal defect had been sutured directly. In this patient his pericardial outflow prosthesis was changed to a prosthesis of teflon. From the experience of this and several other cases we believe that an outflow prosthesis from pericardium is less suitable.

The relative heart volume increased moderately after operation in about half of the material. Wolf et al (24) have earlier shown that cases with outflow prosthesis and pulmonary insufficiency more often get an increase of heart volume. We could not find any definite correlation between heart size and outflow prosthesis in this material. Another explanation of the increased heart volume could be reduced myocardial function by the incision in the right ventricle. Miller et al (20) found left ventricular volumes at the lower normal limit in 12 of 14 cases of non-operated Fallot's disease. It is reasonable to assume that the total correction gives an increased volume of the left ventricle secondary to the increased flow through the lungs.

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ORGAN SPECIFIC INHIBITION OF THE IN VITRO MIGRATION OF LEUCOCYTES IN HUMAN GLOMERULONEPHRITIS

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Abstract The in vitro reactivity of peripheral leucocytes in renal parenchymatous homogenate is examined in 11 normal persons and 34 patients with nephropathia by means of a capillary tube migration technique. In patients with acute glomerulonephritis the migration is inhibited if the culture medium contains a homogenate of fetal kidney. A similar inhibition is not seen with leucocytes from normal persons or patients with terminal nephropathy or pyelonephritis. In brucella hypersensitivity a specific antigen induced inhibition of the in vitro migration of peripheral human leucocytes has been shown in the same experimental system, to be a parameter of cellular hypersensitivity. The reactivity demonstrated in the present study may thus indicate that there exists in acute glomerulonephritis a state of organ specific hypersensitivity of the cellular type directed to antigenic compounds in normal renal parenchyma.

It is generally accepted that some kind of specifically altered reactivity induced by streptococcal antigen is pathogenetically important in acute glomerulonephritis although the mechanism initiating glomerular damage is still insufficiently explained. On the basis of present knowledge an antigen antibody reaction between foreign—possibly glomerulus associated—protein and a specifically reactive immunoglobulin synthesized by the organism may be assumed to cause the initial glomerular lesion (4, 17, 21, 22).

A similar humoral hypersensitivity reaction to foreign antigen however does not satisfactorily explain the progressive renal involvement which may continue for months or years in chronic active states of glomerulonephritis to all appear ance independent of the initially important bacteria. To explain the pathogenetic factors behind this course theories of auto-immunity have been advanced and have found some experimental support (7, 13, 14, 15, 16, 18, 19, 20, 31). According to these humoral as well as cellular hypersensitivity mechanisms may be considered in

strumental in the development of protracted renal damage.

In patients with glomerulonephritis the presence of circulating antibodies with specific reactivity to renal parenchyma components indicates that auto immune mechanisms play some part in the pathogenesis and the deposit of antigen antibody complexes in the glomerular membrane and capillary tufts seems to be an important pathogenetic feature (8, 9). This however does not exclude the possibility that a co-existing organ-specific hypersensitivity of the cellular type may be pathogenetically instrumental (1, 15, 31).

A state of cellular hypersensitivity directed to wards antigenic components of renal parenchyma would be tantamount to the presence in the organism of immunocompetent cells possessing a specific capacity to seek out and react with antigen-containing renal structures. Attempts to demonstrate this type of reactivity in patients with glomerulonephritis by examining the intracutaneous delayed type reaction to human renal extract have not been confirmative (30) and a more exact evaluation of organ-specific cellular reactivity has so far not been possible in human glomerulonephritis mainly because an in vitro method for detection of cellular hypersensitivity in man has not been available.

On the basis of methods developed by George and Vaughan (11) and David et al (5, 6) the author suggested a technique for evaluating the in vitro migration of peripheral human leucocytes. With this technique it could be demonstrated that the in vitro migration of leucocytes from brucella positive persons is specifically inhibited by brucella bacteria (28). Several observations indicate that specific antigen induced inhibition of immunocompetent cell migration is an in

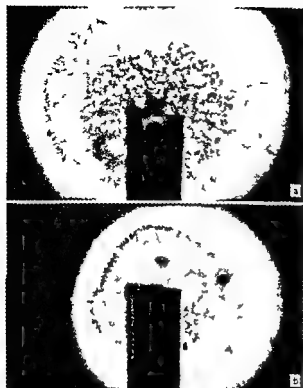


Fig 1 Leucocyte migration culture from case of active glomerulonephritis. Migration period 4 hours (a) Control (b) Inhibition of migration induced by fetal renal homogenate

vitro correlate to cellular hypersensitivity (5, 11, 12, 23, 25, 26, 29) and this has been confirmed also with the *in vitro* technique employed in the present investigation (27, 28). Further the present technique seems so far to permit an evaluation of cellular hypersensitivity in states which can not be estimated by more traditional methods e.g. autoimmune diseases (2).

If there exists in glomerulonephritis a state of cellular hypersensitivity specifically directed to antigenic compounds in renal parenchyme one should expect renal parenchymatous components to induce an inhibition of the *in vitro* migration of leucocytes from patients with active glomerulonephritis. An experiment of this kind might throw new light upon the pathogenesis of glomerulonephritis. Leucocytes from patients with pyelonephritis or states of terminal nephropathia with fibrotic renal tissue might beforehand be expected to behave differently in the test system and the examination might thus find diagnostic applications or be informative concerning the activity of the disease at a given time besides giving information concerning pathogenetic relations.

MATERIAL AND METHODS

Material

1 Eighteen consecutive patients without demonstrable renal affection

2 Fifteen consecutive patients with active glomerulonephritis. Renal biopsies in 14 cases showed proliferative glomerular affection in two membranous glomerular affection in three and co-existing changes of both types in nine cases. Clinically eight patients had a nephrotic syndrome with a 24 hour renal protein loss of more than 3.5 g.

3 Nineteen consecutive patients of whom seven had terminal nephropathia with maximally contracted kidneys of unknown etiology (no histology), seven chronic glomerulonephritis in terminal phase (four biopsically verified), four chronic pyelonephritis and one acute pyelonephritis. Creatinine-clearance was below 20 ml/min in 15 patients and within the normal range in three cases of pyelonephritis.

All diagnoses were made on the basis of generally accepted anamnestic and objective criteria and were unknown to the laboratory performing the leucocyte migration test (LMT).

Antigens

Tissue homogenates were prepared from lyophilized sterile fetal kidney, liver, colonic mucosa and ileal mucosa. All preparations were standardized by simple quantitative protein determination. The concentration of homogenate in the test cultures was with kidney and liver homogenates 160 µg with colonic or jejunoileal mucosa homogenates 80 µg protein per ml culture medium.

Technique

Heparinized blood from a cubital vein is allowed to settle for one hour at 37°C. The plasma with white cells is aspirated and the cells (ca. 60% mononuclear, 40% polymorphonuclear leucocytes) are washed four times in Hanks' BSS and placed in capillary tubes. The cells migrate from the free end of the tube into a 1 ml tissue culture chamber containing TC 199 culture medium with 10% horse serum. After 24 hours the migration is visible as a nearly circular sheet protruding from the tube opening (Fig. 1a and b) and measurable by planimetry in a projection microscope. On the basis of the average 24 hour migration area in a series of control cultures without antigen M_0 and the average 24 hour migration area in a series of cultures containing the antigen M_x the migration index

$$MI = \frac{M_x}{M_0}$$

is calculated. MI thus indicates an inhibition of the cell migration if the values are lower and a stimulation if greater than unity.

RESULTS

The results are presented in Fig. 2. The white cell migration of 18 persons without demonstrable renal affection (left column) appears to be

rather unaffected by the presence of renal homogenate. The normal range (mean \pm 2 SD) of MI is 0.83–1.11. The findings in 15 cases of active glomerulonephritis are indicated in the middle column. Subnormal values of MI are found in 13 cases and the observations show a distribution significantly different from the normal material ($p < 0.001$). Despite three subnormal levels the mean white cell migration of patients with terminal nephropathy or pyelonephritis (right column) does not show any significant difference from the normal material but is significantly different from the group with active glomerulonephritis ($p < 0.001$).

In all cases showing inhibition with renal homogenate MI appeared to be quite uninfluenced by colonic or ileal mucosa homogenates. In two cases of acute glomerulonephritis the leucocyte migration was inhibited by hepatic as well as renal homogenate.

DISCUSSION

In human glomerulonephritis several observations support the view that circulating antirenal immunoglobulin is an important cause of glomerular damage. This does not exclude however that antirenal antibody may at the same time be regarded also as an epiphenomenon secondary to otherwise caused renal destruction. Its very presence indicates that the organism contains immunocompetent cells specifically equipped to synthesize antirenal antibody. It may consequently be supposed that other immunocompetent cells not necessarily engaged in antibody production but with the same specificity might possess the ability specifically to react with antigen-containing renal structures following the pattern of a cellular hypersensitivity reaction.

Experimentally a combined proliferative and membranous glomerulonephritis can be induced in rats by injection of renal extract in Freund's adjuvant (14). In transfer experiments lymphocytes from rats with this kind of experimental glomerulonephritis seem to cause similar glomerular damage in normal recipient animals (13). Transferability by means of lymphocytes has been demonstrated in other kinds of experimentally induced auto-immune diseases (10–24) and is at the same time a fundamental feature of cellular hypersensitivity (3). Although these experiments

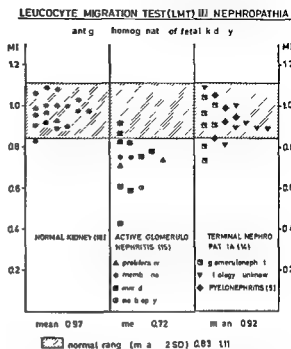


Fig. 2 Leucocyte migration test (LMT) with homogenate of fetal kidney in 18 normal persons and consecutive cases of active glomerulonephritis (15) and terminal nephropathy or pyelonephritis (19). MI = migration index.

are informative only of a special type of glomerulonephritis experimentally induced in rats they seem to indicate that a mechanism of organ specific cellular hypersensitivity can be instrumental in glomerular damage.

From the present study it appears that the *in vitro* migration of leucocytes from patients with active states of glomerulonephritis is inhibited if the culture medium contains a homogenate of fetal kidney. The migration of leucocytes from normal persons is uninfluenced by the homogenate and patients with pyelonephritis or terminal fibrotic nephropathy do not show any significant difference from the normal group. The white cell reactivity to renal tissue components thus indicates a distinct difference between glomerulonephritis and the other renal diseases examined and discloses the presence in normal renal parenchyma of some compound which possesses the ability specifically to inhibit the migration of leucocytes from patients with glomerulonephritis.

The specific antigen induced inhibition of leucocyte migration as studied by the present technique seems to be a reliable parameter of cellular

hypersensitivity (27-28). As to the specific reactivity to renal tissue components observed in the present experiments a similar view may be taken. The results can accordingly be interpreted as indicating the existence in active human glomerulonephritis of a state of organ specific hypersensitivity of the cellular type directed to antigenic components of renal parenchyma. Even if this type of autoreactivity might afford a good explanation of several pathogenetic relations in glomerulonephritis its importance as a pathogenetic factor cannot be evaluated on the basis of the present investigation.

ACKNOWLEDGEMENTS

This study was supported by P. Carl Petersens Fond, F. L. Smidth & Co. A/S's Jubilæumsfond and Statens Almndelige Videnskabsfond.

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SERUM MONOAMINE OXIDASE (MAO) IN DIABETES MELLITUS AND SOME OTHER INTERNAL DISEASES

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Abstract Serum monoamine oxidase (MAO) activity has been studied in normal adults and in about 500 persons with diabetes mellitus and some other internal diseases. We have confirmed earlier reports that serum MAO is increased in patients with chronic congestive heart failure and that the enzyme level is correlated to the degree of incompensation. Increased serum MAO concentration was also registered in persons with thyrotoxicoses and diabetes mellitus. Serum MAO activity was found to be increased already at the first appearance of diabetes mellitus and was not influenced by the type of therapy. A decreased serum MAO activity was found during treatment of different diseases with corticosteroids and ACTH as well as in some cases of neoplastic diseases.

As shown by McEwen and Cohen (5) human blood serum contains a soluble monoamine oxidase that catalyzes the oxidative deamination of several monoamines—i.e. benzylamine, phenylethylamine, tyramine, dopamine and tryptamine. (3) This enzyme is in many respects similar to mitochondrial MAO which however also catalyzes the oxidation of other amines i.e. epinephrine, norepinephrine and serotonin. It might be possible that serum MAO is an isoenzyme of mitochondrial MAO.

It has been suggested that the serum MAO activity is independent of age, sex and meals. Unlike serum diamine oxidase, serum monoamine oxidase is not elevated during pregnancy. (2) In recent years it has been shown that serum MAO activity is increased in chronic congestive heart failure of different origin, (6) in liver cirrhosis, (4) and in diabetes mellitus. (8) In addition the enzyme level has been reported to be increased in a few cases of thyrotoxicosis and carcinoid syndrome. (6) Treatment with nitroglycerin decreases mitochondrial MAO activity. (9) Consequently it might possibly also cause a decrease of serum MAO.

We started this investigation with the general intention to study the variations of serum MAO activity in different clinical conditions. We also intended to find out the value of serum MAO determination as a screening test for chronic congestive heart failure.

MATERIAL

The material consisted originally of all 80 patients hospitalized on one occasion in a male and a female ward of the Department of Internal Medicine. After the finding of divergent serum MAO values in diabetes mellitus, myeloproliferative diseases and thyrotoxicosis as well as under steroid treatment, these special groups of patients have been further completed. With regard to diabetes the large series of the hospital described by Nilsson et al. (7) could be used. The total number of persons investigated is about 500. For comparison with the figures of McEwen the enzyme level has also been determined in 38 healthy blood donors.

METHODS

Serum MAO activity was determined according to McEwen and Cohen (5) with benzylamine as the substrate. Assay and control reaction were incubated in open test tubes at 37°C for 3 hours. The assay tube contained 600 μ l of serum, 700 μ l of 0.1 M phosphate buffer (pH 7.2) and 80 μ l of 6 mM benzylamine hydrochloride (in the same phosphate buffer). At the end of the incubation time 80 μ l of 50 per cent perchloric acid was added to stop the reaction. In the control tube the substrate was added just before the perchloric acid. 15 ml of cyclohexane was added to each tube and the contents of the tubes were emulsified with a "super mixer". The tubes were allowed to stand for 15 minutes at room temperature and were then after a second emulsification, centrifuged in a clinical centrifuge. After that the absorbancy of the cyclohexane extract was measured at 4 nm with a Zeiss PMQII spectrophotometer (10 mm cuvette). An enzyme unit was defined by McEwen as the difference in optical density between

Table I Serum MAO in normal series and in various clinical states

	n	Mean age (y)	MAO U (McEwen)	
			Range	Mean
A Blood donors	38	31.0	13.4-51.5	29.6
In patient not classed below	57	58.8	16.1-52.4	30.5
B Cardiac insufficiency	41	69.0	15.0-91.8	44.2
Degree of incompensation	1	23	15.0-63.6	37.9
2	7		34.3-57.8	43.5
3	8		21.2-91.8	56.5
4	3		57.0-68.2	62.5
Cardiac infarction	15	68.9	21.2-63.6	40.4
First infarction	9	70.8	21.2-49.4	33.1
Nitroglycerin treated	5			46.4
C Arterial hypertension	13	52.5	16.7-49.7	35.4
Treated with α methylidopa	7		32.0-44.9	34.3
D Thyrotoxicosis	8	62.5	39.4-87.7	57.8
E Corticosteroid treated	23	56.8	15.5-47.2	26.1
Without cardiac disease	17		15.5-34.5	22.2
Bronchial asthma	14			25.1
Rheumatic disease	2			16.3
Neurologic disease	1			18.4
F Leucemia malignant lymphoma and myeloma	8	63.6	10.7-42.0	21.2

assay and control multiplied by 100. According to the recommendations of the International Union of Biochemistry on the Nomenclature and Classification of Enzymes a unit (U) is the amount of enzyme that catalyzes the degradation of 1 μ mol of the substrate per minute under well defined conditions. Consequently the

MAO concentration can be expressed in μ U/ml by multiplying the difference in optical density between assay and control by 1200. The figure has been found by determination of the extraction efficiency (about 80 per cent) of benzaldehyde in the procedure and the molar coefficient of extinction 1.4×10^4 l/mole cm (at 242 nm in cyclohexane). The precision of the method in our hands is the same as reported by McEwen and Cohen (5).

The degree of cardiac incompensation has been described by the weight loss of the patient during the stay at the hospital. Degree 1 has been defined as a body weight loss of less than 3 kg, degree 2 between 3 and 7 kg, degree 3 between 7 and 15 kg and degree 4 more than 15 kg.

RESULTS AND DISCUSSION

The results of the serum MAO determinations have been summarized in Table I and with regard to diabetes mellitus in Table II.

A Normal values

Our serum MAO values are in very good agreement with those reported by McEwen and Cohen (5) for the age 18 to 50 years. The values found for healthy blood donors are not different from

those found for in patients of the Department of Internal Diseases or the Department of Surgery without a clinical picture or treatment known to influence the serum MAO activity. A significant increase of serum MAO was however found for children (1).

B Chronic cardiac heart failure

The degree of cardiac incompensation has been roughly estimated by determination of the body weight reduction during therapy. In this way the correlation between the degree of heart incompensation and the serum MAO activity has been convincingly proved. This is a confirmation of earlier results of McEwen and Harrison (6) who used exact hemodynamic measurements for the determination of the cardiac state. When the patients with chronic congestive heart failure were dehydrated there was a tendency to lowering of the serum MAO values. However they were not completely normalized. An attempt was also made to correlate the serum MAO activity and the heart size determined by roentgenological techniques but this correlation was found to be very uncertain. A group of patients with heart infarction was found to have almost normal serum MAO values at the time of the first heart attack. In connection with reinfarction the degree of incompensation as well as the serum MAO level

Table II Serum MAO in diabetes mellitus

	n	MAO U (McEwen)	
		Range	Mean
H Diabetes mellitus	340	20.7-99.4	48.0
Males total	152	21.6-76.4	45.0
Age y 20-39	35	30.6-67.9	45.1
40-59	54	21.7-80.0	44.7
60-79	63	21.6-76.4	45.1
Females total	188	20.7-99.4	50.6
Age y 20-39	27	29.0-61.5	42.3
40-49	19	29.2-86.0	50.3
50-59	29	26.8-99.4	55.2
60-69	67	25.0-88.7	51.0
70-79	46	20.7-84.8	51.9
Insulin therapy	191	21.6-99.4	49.4
Insulin > 60 i.u.	26	23.0-67.9	50.5
Peroral therapy	149	20.7-84.8	46.9
Newly discovered disease	14	29.2-97.0	48.1
> 15 years duration	34	23.0-73.0	50.6

were found to be elevated. Therapy with nitroglycerin did not generally decrease the serum MAO activity. However a few unexpectedly low enzyme values were registered for patients with such treatment. The highest values found for the cardiac patients were 92.0 and 88.7 U (McEwen). These two patients were also diabetics. Two other cardiac patients with exceptionally high values (87.7 and 73.4) were also suffering from thyrotoxicosis.

C Arterial hypertension

In patients with arterial hypertension without known effects on the heart a very slight serum MAO increase was noted. *m*-methyl dopa (0.25 g \times 2-4) was not found to influence the enzyme level.

D Thyrotoxicosis

As earlier reported by McEwen and Harrison (6) we found increased serum MAO values in connection with thyrotoxicosis. The material is yet too small for the determination of a possible correlation between the degree of thyrotoxicosis and the serum MAO level. The lowest enzyme value in this group was registered for a patient with intensive symptoms of thyrotoxicosis factitia. In two cases with extreme myxedema normal serum MAO activity was registered.

E Steroid treatment

In connection with steroid treatment low serum MAO values have been registered with few ex-

ceptions. Prednisolone has been given in doses of 2.5-5 mg \times 1-3 for periods up to 3 years. Treatment with ACTH has been given as occasional therapy in status asthmaticus. In three cases low values about 20 U (McEwen) were found already 2-3 days after treatment with ACTH (dose = 60 IU \times 3) had started. In a small subgroup of steroid treated patients with electrocardiographic signs of heart disease the MAO values were not especially low.

F Malignant diseases

In a few cases of leucemia, myeloma and malignant lymphoma the serum MAO concentration was decreased to excessively low values.

G Nephrosis and myasthenia gravis

Apart from the cases included in Table I there are two cases of special interest.

Case 1 A woman 33 years old with nephrosis which appeared two months after a throat infection. During treatment with ACTH the proteinuria was reduced from 0.04 g/100 ml to 0.01 g/100 ml. Serum MAO activity decreased from 106.2 U (McEwen) which is the highest value yet registered by us to 88.5 U (McEwen) after 10 days treatment and to 77.2 U (McEwen) after 20 days. However it must be mentioned that another patient with a similar clinical picture of nephrosis had normal serum MAO values.

Case 2 A woman 37 years old with a newly diagnosed myasthenia gravis initially had a serum MAO value of 54.6 U (McEwen). The enzyme level was unchanged after two months during which period the patient was successfully treated with mestonin. Another case of myasthenia gravis a woman 38 years old had a serum MAO value of 39.5

II Diabetes mellitus

In diabetes mellitus there is a statistically significant increase of serum MAO concentration (8). Only 14 of the 340 persons in the diabetic group had serum MAO values below 30 U (McEwen) which was the normal mean value. The diagnosis of diabetes mellitus was dubious in eight of these 14 patients because it was founded only on single pathological glucose tolerance tests or on solitary increased blood sugar values. Only two of the 95 control persons (group A) in this study had a serum MAO activity higher than 48 U (McEwen) which was the mean for the diabetics. The increase seems to be independent of the duration of the disease. In six patients with diabetes mellitus the increased enzyme value was registered already when the disease was first detected and diagnosed. The serum MAO activity seems independent of the type of treatment and the dose of insulin and there seems to be no correlation to the development of complications. Occasional insulin treatment for example in psychiatric diseases was not found to influence the serum MAO level. Diabetics with chronic congestive heart failure or thyrotoxicoses were found to have especially high enzyme values. Further studies on the correlation between serum MAO and glucose metabolism are in progress with special regard to diabetic gene carriers.

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LACTIC DEHYDROGENASE IN KIDNEY TISSUE AND RENAL DISEASE

Adaptive Change of the Synthesis in Acute Renal Failure

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Abstract The concentration of lactic dehydrogenase (LDH) and LDH isoenzymes was determined in different zones of normal human kidneys and in sera from patients with renal diseases.

The isoenzyme pattern in renal tissue was characterized by an increasing relative concentration of the M subunit from the cortex to the papilla. The outer non-glomerular cortex corticis zone presented a medulla-like isoenzyme pattern which has not been described hitherto. This correlated well with new data on renal blood flow in this zone.

Patients with chronic renal failure had normal or slightly elevated serum concentrations of LDH and the isoenzyme pattern was inconclusive.

In patients with renal trauma, serum LDH concentration was very high but the isoenzyme pattern did not differ from the normal.

In acute oliguric glomerulonephritis serum LDH was moderately elevated while LDH concentrations were usually very high in acute renal failure of widely different pathogenesis. In both groups however the isoenzyme pattern exhibited a uniform relative increase of the M-subunit concentration. Taking into consideration new data on (a) renal blood flow in acute renal failure and (b) adaptation of LDH synthesis to varying oxygen tension the hypothesis is advanced that the isoenzyme pattern reflects an altered synthesis of LDH in the kidneys as an adaptive response to hypoxia in the initial phase of acute renal failure.

This report deals with studies of lactic dehydrogenase (LDH) and LDH isoenzymes in different zones of normal human kidney tissue and in serum from patients with acute and chronic renal diseases.

In a previous paper (14) we reported on a considerable increase of LDH in serum from patients with acute renal failure. We also demonstrated a uniform increase of all five isoenzymes consistently with the pattern obtained from whole kidney tissue homogenate.

LDH is an intracellular enzyme in the energy

metabolism and consequently it is found in almost all human tissues. The concentration of LDH (units per gram tissue) however varies considerably in different tissues. Apart from the retina the highest concentration is found in the kidney (40).

In normal serum the concentration of LDH and the isoenzyme pattern is supposed to be the result of a release of enzymes from different tissues. Pathological states in tissues with a low concentration of LDH resulting in an increase of the serum concentration indicate a rather extensive cell lesion (i.e. necrosis or altered membrane permeability) as a liberation of 10 000 LDH units is required to cause an elevation of the concentration of 1 unit per ml in an extracellular volume of approximately 10 litres. In tissues with a high concentration of LDH a far less extensive cell lesion would give the same result. A change in the isoenzyme pattern in serum further requires that the isoenzyme pattern of the enzyme liberating organ differs considerably from that of serum in which case even minor contributions may cause a demonstrable change in the pattern of serum thus making the isoenzyme determination a more sensitive test than the total LDH determination (e.g. the protracted elevation of LDH₁ after myocardial infarction or of LDH₂ in subclinical or protracted hepatitis despite normal total activity (39)).

During recent years the theory (1) of two types of LDH (H_4 and M_4) tetramerically constituted from two different monomers or subunits (H and M) has received great support from divergent physico-chemical properties (11-37). By hybridization of the two subunits into tetrameric molecules another three intermediate fractions

are formed (H_3M , H_2M_2 and HM_3) (23) thus resulting in five electrophoretically separate proteins or isoenzymes. The biologically most fundamental distinction between the two subunits appears to be the influence of the oxygen tension (pO_2) on the rate of synthesis of the subunits. Experiments have shown that a low pO_2 in homozygous cell culture promotes the synthesis of the M subunit while a high pO_2 results in a preferential synthesis of the H subunit (4, 6, 8). On the basis of these data the Kaplan group advanced the *oxygen tension theory* according to which the rate of synthesis of the single subunits in cells is determined by the local pO_2 . This corresponds well with the observations that H_4 LDH works optimally under aerobic conditions with a low concentration of lactate while M_4 LDH is inhibited only by very high concentrations of lactate thus preserving its catalytic activity also under anaerobic glycolysis (11).

In this report the hypothesis is advanced that the synthesis of LDH in kidney tissue is altered as an adaptive response to hypoxia during the initial phase of acute renal failure (syn. ischaemic nephropathy, acute tubular necrosis).

METHODS

The serum concentrations of LDH and glutamic pyruvic transaminase (GPT) were determined by fluorometric methods (18, 19). The enzyme concentration was expressed as units per ml. One unit was defined as the amount of enzyme which was capable of causing a reaction velocity of 1 μ mol per hour. Normal values were LDH = 7–21 units per ml (168 persons) and GPT = 0–1 μ units per ml (172 persons).

The determination of LDH isoenzymes was performed according to a technique originally described by Wieme (38) and later modified by Jensen and Laursen (10). Agarose was used as a supporting medium and NADH and pyruvate were used as substrate. The relative distribution of the five isoenzymes was calculated by weighing the single fractions of the curve obtained by photometric scanning of the electrophoresis plate. The total concentration of the H and M subunit as defined by Kaplan and Goodfriend (11) was calculated from the percentage distribution of the five isoenzymes. The percentage of the total M subunit will be referred to as the MSU value. The standard deviation on the difference between

double determinations of the MSU value in 20 pathological sera was 2.8%.

The reliability of this method was checked by another more laborious quantitative method based on an extraction of the single isoenzymes followed by a quantitative measurement of the concentration in test tubes (20). In this method a standard deviation of 0.9% was obtained on differences between 13 double determinations. In nine sera both methods were applied. The standard deviation on differences between the MSU values (ranging from 20 to 65%) was 3.9%. This difference was insignificant ($P > 0.05$).

Sera from 30 healthy males between the ages of 20 and 30 years served as normal material. Results appear from Table II.

Kidney tissue preparation

Samples of human kidney tissue were cut into pieces and carefully rinsed in 0.9 per cent saline. After homogenisation (400 rpm in a Potter Elvehjem homogenisator for 2 min at 0°C) and centrifuging (at $2500 \times g$ for 10 min) the LDH isoenzymes in the supernatant were determined according to the above mentioned technique.

MATERIAL

Twenty one samples of human kidney tissue were obtained from seven kidneys at autopsy (12–15 hours post mortem). There was no evidence of renal disease prior to death and the autopsy did not reveal any pathological features in the examined kidneys.

Three samples were cut tangentially in thin slices (1–2 mm) from the cortex corticis zone after removal of the fibrous capsule. Nine samples from the cortical and six samples from the medullary (= non glomerular) zones were cut in varying depth. Finally three samples were cut from the apex of the papilla after removal of connective tissue.

Sera were examined in 74 patients admitted to our renal unit with different renal diseases. The material was divided into four groups:

- A Acute renal failure 44 patients
- II Renal trauma 8 patients
- C Acute oliguric glomerulonephritis 4 patients
- III Chronic renal failure 18 patients

In groups A, II and C several determinations of LDH were made within the first week after

Table I The concentration of lactic dehydrogenase and the relative distribution of isoenzymes in different zones of the kidney

Kidney zone	n	Total LDH (unit/ml)	LDH isoenzymes (%)					M-subunit ()	P
			I	II	III	IV	V		
Cortex corticis	3	265 (228-340) ^a	30	30	21	13	6	34±1.4 ^b	<0.005
Cortex	9	254 (60-38)	38	32	17	8	5	27±4.7	
Medulla	8	292 (112-387)	27	27	20	16	10	39±4.8	<0.001
Papilla	3	204 (82-293)	13	19	22	24	22	55±3.5	<0.005

^a Mean value and range in brackets^b Mean value ± one standard deviation.

admission and the MSU values were calculated from the maximal LDH concentration recorded. This was in every case the first value obtained.

A Acute renal failure All patients fulfilled the diagnostic criteria of acute renal failure cited in our first report (14). The patients were subdivided into six groups as follows:

I Posttraumatic (7 patients) Acute renal failure following traffic accidents. Various and often widespread extrarenal lesions were present in all patients.

II Gastrointestinal tract surgery (8 patients) Precipitating causes were: Perforating acute appendicitis (four), perforation of gastroduodenal ulcer (three) and removal of rectal cancer (one).

III Biliary tract disease (7 patients) Anuria followed cholecystectomy.

IV Other surgical causes (7 patients) Anuria followed other types of major surgery (vascular and lower urogenital tract surgery).

V Infections (9 patients) This heterogeneous group comprised patients in whom severe infections were the dominant or the only precipitating cause.

VI Other medical causes (6 patients) Primary disorders were: Barbiturate intoxication, haemolytic anaemia, transfusion reaction, dehydration, diabetic coma and eclampsia.

B Renal traumata Eight anuric patients are grouped under this heading as all of them presented necrosis of renal tissue in varying degree. Only two of the patients had extrarenal lesions as well, both following traffic accidents. Their clinical course was indistinguishable from that of

acute renal failure, but autopsy revealed severe cortical infarction with widespread necrosis. An other four patients showed uni- or bilateral renal infarctions and necroses at autopsy as a result of renal artery occlusion. Two patients, survived one otherwise healthy patient was referred to us with post renal obstruction after accidental perforation of both kidneys (ureteral catheterisation). This patient was included in our first report (14). The other patient underwent nephrostomy in his single kidney because of post renal obstruction. LDH was determined the day after this operation.

C Acute glomerulonephritis Four patients developed oliguria in the initial phase of acute glomerulonephritis. Glomerular filtration rate was severely decreased. The diagnosis was ascertained by biopsy.

D Chronic renal failure Eighteen patients had severe uraemia due to chronic glomerulonephritis (ten) and chronic pyelonephritis (eight). Many of them were in end stage renal failure and some were referred to us with oliguria of uncertain genesis.

RESULTS

LDH in human kidney tissue homogenate

The average total LDH concentration and the mean distribution of the five isoenzymes in four different zones of the human kidney are shown in Table I. All isoenzymes were easily identified in all tissue samples. But, as appears from the calculated MSU value, a significant difference in the relative distribution was observed between the single zones.

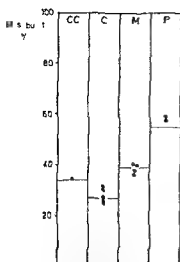


Fig. 1 The relative M subunit concentration in 21 samples of kidney tissue from cortex corticis (CC) cortex (C) medulla (M) and papilla (P). The horizontal lines indicate the mean value of each group.

The single values are presented graphically in Fig. 1. The lowest MSU values were obtained in the cortical zone and the M subunit showed an increasing predominance when moving from the cortex towards the papilla. Surprisingly the MSU value of the cortex corticis zone turned out to be significantly higher than that of the underlying cortical zone ($P < 0.005$) whereas no difference was observed between cortex corticis and medulla ($P > 0.05$). This feature hitherto not recognized will be further commented on in the discussion.

LDH in serum

A. The LDH concentration was elevated in all patients with acute renal failure and in most of them the values were high (above 100 units/ml) (Table II). The variation observed within the single group as well as between the groups may be somewhat exaggerated by the fact that values obtained do not represent true maximal concentrations as patients were received at various intervals from the onset of renal failure and the concentration has been shown to fall rapidly within the first week (14). It was repeatedly observed that serum LDH fell continuously uninterrupted by further traumatizing therapeutic or complicating events in the course of the disease. Neither could we detect any influence of rapid change in the serum urea level provoked by haemodialysis. The typical course in two severe cases appears from Figs. 2 and 3.

The concentration of all five isoenzymes was often markedly increased. The distribution of the calculated MSU values was significantly higher than that of the normal material ($P < 0.001$). From the uniform distribution of the isoenzymes it appears that the high MSU values were not dependent on the increase of any single isoenzyme especially the fifth which might be liberated from e.g. the liver. To test this possibility we correlated serum GPT to LDH 5 and the MSU value. As seen from Fig. 4 no correlation was observed. High values of LDH 5 and

Table II The concentration of lactic dehydrogenase and the relative distribution of isoenzymes in normal persons and patients with renal diseases

	n	Total LDH (units/ml)	LDH isoenzymes (%)					M subunit (%)	P
			I	II	III	IV	V		
Controls	30	16 (7-21) ^a	23 ± 6.0 ^b	41 ± 5.8	24 ± 5.0	5 ± 4.5	8 ± 4.3	34 ± 6.0	
Acute renal failure	44	120 (2-676)	20	26	20	14	20	47 ± 9.8	<0.001
I Posttraumatic	7	217 (84-676)	21	25	19	18	21	47 ± 9.8	
II Gastro intestinal tract	8	91 (50-149)	19	26	21	17	22	47 ± 14.7	
III Biliary tract	7	33 (22-46)	15	30	1	14	19	48 ± 8.1	
IV Other surgical cases	7	87 (50-160)	22	26	22	12	17	44 ± 6.2	
V Infections	9	161 (51-326)	21	23	19	17	21	48 ± 9.4	
VI Other medical cases	6	126 (41-270)	21	28	17	14	20	46 ± 17.0	
Renal trauma	8	166 (31-305)	25	28	21	12	13	40 ± 5.7	0.05 $p > 0.01$
Acute glomerulonephritis	4	42 (28-53)	17	30	21	16	16	47 ± 1.5	<0.001
Chronic glomerulonephritis	10	29 (13-55)	24	31	22	12	11	38 ± 7.7	n.s.
Chronic pyelonephritis	8	24 (17-30)	17	26	19	18	23	50 ± 12.2	<0.001

^a Mean value and range in brackets

^b Mean value ± one standard deviation

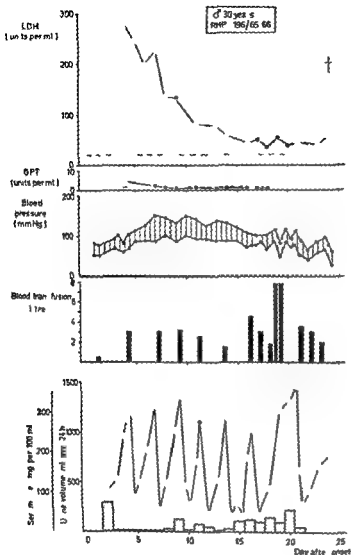


Fig 1 Serum lactic dehydrogenase (LDH) and glutamic pyruvic transaminase (GPT) correlated with the clinical course in a patient with acute renal failure following severe pancreatitis. Haemodialysis was performed seven times. The steep rise of the serum urea concentration between each haemodialysis reflects the dramatic course dominated by severe intra abdominal necroses and gastrointestinal bleeding.

MSU were often seen despite normal or only slightly elevated GPT and even high GPT concentrations were not accompanied by any remarkable increase of the other variables.

The most remarkable finding however was the almost identical average MSU value of around 47% obtained in all six subgroups of patients. The observed values are thus apparently independent of the precipitating causes leading to renal failure and of the great variation in the maximal LDH concentrations recorded. This fact further speaks in favour of a common origin of LDH as will be discussed later.

B Renal trauma In these patients with documented lesion of renal tissue the maximal LDH

concentration was well correlated with the degree of cell damage. In five patients with severe uni- or bilateral necroses in the kidney the LDH concentration was extraordinarily high (max. LDH = 305, 300, 227, 210 and 150 units per ml). In one patient multiple microscopic cortical infarctions were found (max. LDH = 97 units per ml). Two patients survived from minor and localized renal lesions (max. LDH = 42 and 31 units per ml). The LDH concentration showed the same uninterrupted fall during the course of the disease as seen in acute renal failure.

Although not significantly different from the normal, the high mean MSU value of the group may be somewhat misleading as in fact only the two patients in whom renal failure compli-

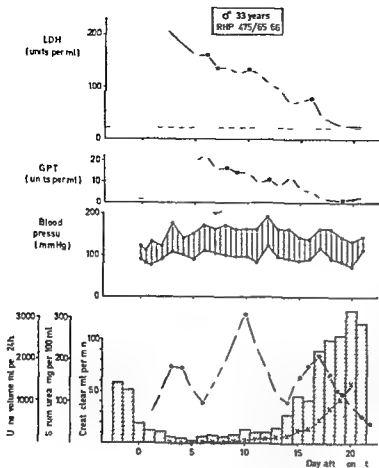


Fig 3 Serum lactic dehydrogenase (LDH) and glutamic pyruvic transaminase (GPT) correlated with the clinical course in a patient who recovered from acute renal failure following bilateral thromboendarterectomy of the femoral arteries. Peritoneal dialysis was performed twice. From the 7th day the patient developed progressive necroses in both legs necessitating bilateral femoral amputation on the 16th day.

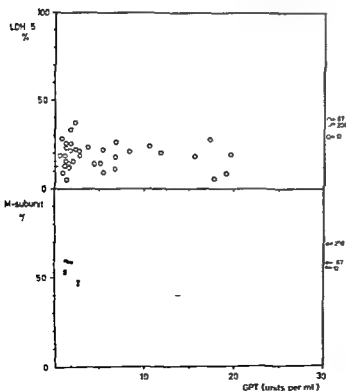


Fig 4 Relationship between the serum concentration of glutamic pyruvic transaminase (GPT) and the relative concentration of the fifth isoenzyme (LDH 5) and the M-subunit. The dashed lines indicate upper normal limits. As is evident from the figure no correlation exists.

cated traffic accidents had significantly elevated MSU values (45 and 52°) In the other six patients values were near normal (mean = 37.6°)

C In patients with *acute oliguric glomerulonephritis* the serum LDH concentration was moderately increased (Table II) The isoenzyme pattern however turned out to be identical with that of acute renal failure Despite the small number of patients this observation is probably reliable as the standard error of the average MSU value is very low ($s.e. = 0.8$) The mean MSU value differed in a highly significant degree from that of the normal material ($P < 0.001$)

D In 14 of the 18 patients with *chronic renal failure* serum LDH was slightly elevated The isoenzyme patterns were indeed very inconclusive as is evident from the standard deviations However when the average MSU value of chronic glomerulonephritis is compared with that of chronic pyelonephritis a systematic difference arises although only significant at the 5% level ($0.05 > P > 0.025$)

DISCUSSION

Human kidney tissue homogenate

The presence of five isoenzymes in the kidney was described in rats by Wieme and Van Maercke (39) Our results which show an increasing relative concentration of MSU from the cortex towards the papilla confirm earlier observations in rats (28) and in rabbits (34) In human kidney Mattenheimer found MSU values corresponding to ours (calculated from data cited by Ringoir (29))

This shift from an aerobic to an anaerobic isoenzyme pattern corresponds well with the demonstration of a falling pO_2 gradient over the kidney (2, 21) Kean et al (12) demonstrated a significantly higher rate of anaerobic glycolysis in the inner medulla and a correspondingly higher oxygen consumption in the cortex Significantly decreasing blood flow rates over the three zones were demonstrated by Thorburn et al (33) Thus the MSU values are well correlated with the type of metabolism in agreement with the oxygen tension theory

The significantly higher MSU values in the cortex corticis zone than in the underlying cortical zone was therefore most unexpected We do not believe this could be due to an artefact since

the site of excision was very well defined and the standard error of the average MSU value from different kidneys was small ($s.e. = 0.99$) Values may be somewhat disfigured by the fact that samples were taken from kidneys at autopsy but this would probably affect all zones and therefore does not influence the relative proportion between the values We have not been able to find any account of this observation in the literature but a tendency is apparent from the data given by Thorling and Jensen (34) on LDH isoenzymes in different zones of rabbit kidneys While the mean MSU value showed a linear decrease from the papilla over the inner and outer medulla to the inner cortex zone the MSU value in the outer cortex zone turned out to be slightly higher than in the inner cortex zone (21.1° and 19.9° respectively) Referring to our results this slight increase may be due to a high MSU value in the cortex corticis zone That they did not obtain an even higher MSU value is no doubt due to the fact that their outer cortex samples included tissue from both zones

Our results receive support from data on renal blood flow measured by Ladefoged (17) Renal blood flow in the most superficial part of the kidney as calculated from the disappearance curve of β ray emission of $^{133}\text{Xenon}$ was significantly lower than cortical blood flow rate calculated from the disappearance curve of the γ ray emission of $^{133}\text{Xenon}$ which also reflects deeper parts of the kidney His data were confirmed by autoradiography revealing a considerably delayed washout of $^{133}\text{Xenon}$ in a peripheral cap closely corresponding to the cortex corticis zone

A correlated study of divided blood flow and isoenzyme pattern in the two zones is in progress

The similarity of isoenzyme pattern and blood flow in the cortex corticis and the medulla of the kidney may morphologically and functionally be correlated with the presence of distal tubules in both zones

In conclusion the isoenzyme pattern of the cortex corticis zone apparently does not disprove the oxygen tension theory

LDH and isoenzymes in serum

In *chronic renal failure* the degeneration of renal tissue is usually slow and measurable liberation of total LDH from the kidney would be expected only in acute exacerbations or active infections

Almost all values were normal or borderline in this material and so were the results of Ringoir (29). West and Zimmermann (36) found elevated LDH in 43 of their 71 patients. LDH was not correlated to the degree of azotaemia. Jahnecke et al (9) did not detect any rise of LDH.

As our material consists of patients with normal and moderately elevated serum enzyme concentrations, the considerable spread of MSU values is not surprising. Yet the high mean value of MSU in pyelonephritis and the low mean value in glomerulonephritis corresponds well with the site of lesion and the isoenzyme pattern in the medullary and the cortical kidney zones, an observation also made by Ringoir (29).

The elevated serum LDH in *acute oliguric glomerulonephritis* and more so in *renal trauma* indicates altered membrane permeability and/or necrosis of renal tissue. Considering the slight difference between the MSU value in normal serum and the kidney zones involved, a significant change of the isoenzyme pattern in serum could hardly be expected. Accordingly, normal values of MSU were found in six of the patients with renal trauma, while surprisingly two of the traumatic patients and all four patients with acute glomerulonephritis showed elevated values. This may be due either to extrarenal lesions or to adaptive change of renal LDH synthesis as discussed below.

The high LDH concentration and the elevation of all five isoenzymes in serum from patients with *acute renal failure* confirm our previous findings (14) and have also been observed by others (9, 29, 36).

The important question arises to what extent is LDH liberated from *extrarenal* tissues owing to cell necrosis and/or shock induced hypoxia in the tissue during the preceding events leading to acute renal failure.

The elevated concentration of LDH 1 and 2 might result from severe haemolysis or myocardial infarction. This probably explains the low MSU value of 29% in one patient with acute renal failure following severe haemolysis (haemolytic crisis). Otherwise none of the patients presented evidence of preceding myocardial infarction or haemolysis.

The dominant and uniform increase in the concentration of M subunits throughout the material might be due to liberation of LDH from the liver

and muscles considering the high concentration of LDH 5 (and 4) in these tissues. A high veno-arterial difference of LDH concentration in *hepatic femoral* and *renal* vessels has been demonstrated in dogs which succumbed from *sustained deep* and *irreversible* haemorrhagic shock (35).

However, in many of our patients no established shock was recorded and deep protracted shock was rare and never irreversible in the initial phase. Hypotensive crises during the course of renal failure or irreversible shock leading to death did not provoke a second rise of the LDH concentration (Fig. 2). Similarly, extensive necroses in both legs eventually leading to bilateral femoral amputation in one patient did not interrupt the continuous fall in the concentration of LDH (Fig. 3). In experimental endotoxin shock, LDH and glutamic oxalacetic transaminase (GPT) both present in high concentrations in the kidneys were considerably elevated while creatine phosphokinase (muscle) was unaltered (15). Furthermore, serum ornithyl-carbamyl transferase (liver) and creatine phosphokinase (muscle) were within normal limits despite a marked increase of LDH 5 in patients with acute renal failure studied by Ringoir (29). Yet it is a common clinical experience that some patients with acute renal failure are slightly icteric despite the lack of evidence of infectious or toxic affection of the liver. This so-called 'shock liver' may give rise to some elevation of GPT. But with few exceptions the majority of our patients showed either normal or only moderately elevated GPT concentrations (< 10 units per ml) and there was no correlation with either the *relative* or the *absolute* concentration of LDH 5 or M subunit in our material. Furthermore, the high MSU value was certainly not dependent on any prominent or isolated elevation of LDH 5 which might derive from the liver. In individual patients with extensive traumatic cerebral necroses we recently observed moderately increased concentrations of LDH (max 71 units/ml) with a rather uniform distribution on the five isoenzymes. However, as none of our patients showed autopsy or clinical signs of cerebral lesions we consider this source improbable in our material.

In conclusion we believe that LDH is liberated to some extent from extrarenal tissue, especially the liver, in acute renal failure. On the other

hand this can only explain a minor part of the total concentration of LDH and, with the exception of a few special cases there is no evidence of any significant influence on the M subunit concentration recorded

We must therefore assume that LDH is mainly liberated from the kidney and that the isoenzyme pattern and hence the MSU value is determined by this source. The kidney has a very high concentration of LDH and considering its isoenzyme pattern it is the only single organ that might cause the observed isoenzyme pattern in serum.

Liberation of LDH from the kidney has been demonstrated in experimental haemorrhagic shock (13, 35) and selective renal infarction or ischemia in experiments on dogs was followed by a prompt elevation of plasma LDH together with a more gradual increase of LDH in urine (3). Elevated LDH in urine has been observed in acute renal failure in man (31). Similar unpublished observations were frequently made in connection with our material and urine isoenzyme electrophoresis often showed elevation of all five fractions. Furthermore the observation of an almost identical MSU value in the six pathogenetically different subgroups and the continuous fall in total LDH concentration in serum uninterrupted by additional traumatizing complications strongly indicates a single dominant source which for the reasons stated above must most evidently be the kidney.

The isoenzyme pattern

When the high MSU values obtained in acute renal failure and acute oliguric glomerulonephritis are compared with those of the different zones in the kidney it becomes apparent that one would have to assume the existence of a lesion mainly located in the inner medulla and the papilla. This is quite inconceivable from a morphological and functional point of view. Maintaining that a significant extrarenal contribution of M subunits can generally be excluded we can therefore only assume that the renal LDH has been subjected to some modification before it is liberated to the serum.

Accordingly we have advanced the hypothesis that the synthesis of LDH in kidney cells is adapted for a preferential production of the M subunits in acute renal failure (25). This adaptation probably takes place within the first 24-48

hours and is mainly restricted to the renal cortex. The adequate stimulus for this adaptation is assumed to be low oxygen tension in the cortical tissue during the initial phase of acute renal failure.

The evidence of differential synthesis of H and M subunits in cells according to the prevailing oxygen tension has been presented above. Adaptive change of LDH synthesis within the same tissue provoked by a change of oxygen tension has frequently been observed in vitro (4, 8) and in vivo (6, 7). That a demonstrable adaptation of the LDH synthesis in vivo is possible within 24 hours has been shown by Thorling and Jensen (34) who submitted rabbits to low oxygen tension for 24 hours and obtained a significant increase of the M subunit concentration in the renal cortex, the zone most susceptible to hypoxia. Finally direct evidence in support of our hypothesis of adapted LDH synthesis in the renal cortex in acute renal failure has recently been provided by the data reported by Ringoir (30) showing a significant increase of the M subunit concentration in renal biopsies in the acute stage of the disease. His conclusion from these data was similar to ours.

It has been much disputed whether low oxygen tension is present in renal tissue in the acute oliguric state. As shown by Thaysen et al. (32) the normal renal cortex has a high oxygen consumption which can be divided into a main functional (or "suprabasal") fraction covering the energy consumed by the active sodium absorption in the proximal tubules and a small basal fraction to cover the oxidative metabolism of the cells under basal conditions, i.e. in the non-absorbent state. This basal oxygen consumption amounts to $1 \mu\text{mol O}_2/\text{g}/\text{min}$ and hypoxia is only present when this oxygen supply is not available. In acute renal failure renal blood flow has been shown to be reduced to about one third of normal (24). Oxygen consumption is proportionately reduced as arteriovenous oxygen extraction is rather constant down to a certain lower limit of renal blood flow (16, 22, 24). Thus this oxygen supply must be regarded as sufficient to cover basal metabolism in the non-absorbent kidney and can hardly condition an adequate lowering of oxygen tension to provoke an adaptive change of LDH synthesis.

However in the very early phase of acute

renal failure Pedersen et al (26-27) succeeded in measuring renal blood flows down to *one sixth* of normal and other available data on arteriovenous oxygen differences with such low blood flows (16) make it highly probable that true hypoxia exists after all. This acute hypoxia may account for the excessive release of intracellular LDH owing to impairment of cellular integrity (not necessarily cell necrosis) as well as the adaptive change of LDH synthesis observed.

Although very few data exist on renal metabolism in acute oliguric glomerulonephritis it is tempting to draw a parallel between the serum isoenzyme pattern in that condition and that of acute renal failure. Cargill and Hickam (5) observed a highly significant reduction of oxygen consumption in acute glomerulonephritis which was closely correlated with the decrease of the glomerular filtration rate (GFR) and Ladefoged (17) measured decreased renal blood flow in subacute glomerulonephritis similarly correlated with the decrease of GFR. Recently he found the renal blood flow decreased to 0.6 ml/g/min in a patient with renal failure due to acute glomerulonephritis. This value is of the same order as those obtained in the initial phase of acute renal failure. GFR was close to zero in our patients and it may be assumed that true hypoxia was present in the acute oliguric state thus accounting for the high serum concentration of the M subunit in this pure renal disease.

CONCLUSION

The results of this study are in agreement with the concept of a low oxygen tension due to reduced renal blood flow in *acute renal failure*. Although they do not allow any conclusions as to the pathogenesis of the initial shut-down of renal function they suggest the following sequence of events:

1 In the initial phase the integrity of renal cells is impaired by the acute hypoxia, resulting in a massive release of presumably unmodified intracellular LDH.

2 In the course of one or two days (perhaps hours) LDH synthesis is gradually adapted to the hypoxic state evidenced by the relative increase of the M subunit in renal tissue and serum.

3 Basal energy metabolism is gradually re-established possibly based in part on anaerobic

glycolysis. Hence the cellular integrity recovers and the liberation of LDH ceases as seen from the gradual decline of serum concentration.

To support this hypothetical model many features still have to be elucidated. Enzyme analysis in tissue and serum correlated with other parameters may in future investigations throw further light especially on the acute phase of the disorder.

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THE EFFECT OF NICOTINIC ACID ON THE DIURNAL VARIATION OF THE FREE FATTY ACIDS OF PLASMA

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Abstract In four patients with hyperlipemia the diurnal variations of plasma FFA were studied before and during, nicotinic acid treatment. It was found that the mean plasma FFA level was higher during, than before treatment with nicotinic acid although a reducing effect on the plasma triglycerides and plasma cholesterol concentrations was observed. The hypothesis that the lowering effect of nicotinic acid on the plasma lipids is secondary to an inhibition of the FFA mobilization from adipose tissue is, reasonably not valid.

It has long been known that long term nicotinic acid administration exerts a lowering effect on plasma cholesterol and total fatty acids (1-7). It was subsequently shown by Carlson and Oro (3) in acute experiments that nicotinic acid depressed the level of plasma free fatty acids (FFA) and Carlson (2) demonstrated that this was due to an inhibition of the lipolysis in adipose tissue. Carlson and Oro (3) suggested that the effect on other plasma lipids might be secondary to a reduced transport of fatty acids from the adipose tissue to the liver. However the lowering of FFA by nicotinic acid was followed by a rise exceeding the original level. The duration and extent of this increase of FFA was not studied in detail. Recently this phenomenon was studied after intravenous injection of nicotinic acid (9). A very impressive increase of FFA was noted between 2 and 4 hours after the administration of nicotinic acid.

The present study was started in order to elucidate the effect of therapeutic doses of nicotinic acid on the diurnal variation of plasma FFA. Frequent recording of the plasma FFA level was feasible with a capillary method for the analysis of FFA (6) which gives results close to the arterial level.

METHODS

FFA was determined according to Laurell and Tibbling (6). Plasma was separated from the blood samples within 10 min. Triglycerides were determined according to Laurell (5) and cholesterol according to Ness et al (8). The patients were given a highly standardized diet, containing 63 g of fat, 104 g of protein and 158 g of carbohydrates daily on both admissions. (4) Breakfast served at 8.30 a.m., luncheon at 12 a.m. and dinner at 5 p.m. No other food was allowed.

Nicotinic acid (Nicagan®) tablets were administered at 8 a.m., 2 p.m. and 9 p.m.

CASE REPORTS

Case 1

Woman, aged 41, two years previously myocardial infarction, when elevation of plasma cholesterol value was observed. Blood samples from relatives were not analysed as they live outside Sweden. She was admitted in December 1966 when the first examination in the present study was made. After this (first) admission she was given nicotinic acid (Nicagan®) 0.5 g three times daily for about one month and was then reexamined. Owing to side effects of the drug the patient refused to take the full, recommended therapeutic dose.

Case

Man, aged 42, suffered from pectoral angina for about three years. His mother and two of her brothers had died aged about 40 of myocardial infarction.

For the present study the patient was first examined in November 1966 when hypercholesterolemia was diagnosed. He was then given nicotinic acid (Nicagan®) in increasing doses up to 1 g three times daily before reexamination about two months later. Only slight side effects of the drug were noted.

Case 3

Man, aged 56 myocardial infarctions in 1965 and 1966. Hypercholesterolemia and hypertriglyceridemia were diagnosed in 1966. In the present study the patient was first examined in June 1967. No heredity traits of interest were observed.

Table I Plasma lipids before and during treatment with nicotinic acid

Nicotinic acids — = before treatment + = during treatment Cholesterol and triglycerides fasting value FFA mean value calculated from the integrated diurnal curves

Case no	Nicotinic acid	Cholesterol (mg/100 ml)	Triglycerides (mM)	FFA mean (mEq/l)
1	—	376	1.26	0.38
1	+	296	0.63	0.92
2	—	446	0.68	0.43
2	+	340	0.47	0.67
3	—	460	4.34	0.57
3	+	400	2.12	0.62
4	—	512	0.85	0.77
4	+	412	0.72	1.09

Nicotinic acid (Nicangin®) was then given in rising doses up to the full dose of 1 g three times daily. About one month later he was reexamined.

Case 4

Man aged 27 known to have essential familial hypercholesterolemia since 1966. Admitted for the present study in March 1967. After this (first) examination patient was given nicotinic acid (Nicangin®) in increasing doses up to 1 g three times daily without any troublesome side effects. He was reexamined about two months later.

RESULTS

As shown in Table I the mean level of FFA during 24 hours was not depressed in any of the patients but strikingly elevated in the majority during administration of nicotinic acid. The mean increase was 0.29 mEq/l. Nonetheless both triglycerides and cholesterol were effectively de-

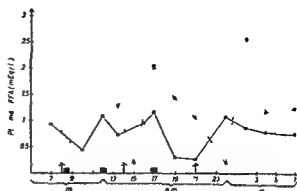


Fig 1 Diurnal plasma FFA level in case 4 without nicotinic acid (unbroken line) and during administration of nicotinic acid (broken line). The nicotinic acid tablets were given at times indicated by arrows and the meals were served at times indicated by squares.

pressed (Table I). During daytime (Table II and Fig 1) an FFA depressing effect of nicotinic acid alternated with periods of increased FFA concentration. The net result of nicotinic acid on FFA level during daytime is rather slight. On the other hand all the patients showed a striking increase in FFA during the night.

DISCUSSION

The alternatively depressing and increasing effect of nicotinic acid on the FFA level corresponds to the findings after a single dose (3, 9). As the increasing effect is strikingly dominant during the night the total result is an increase of the mean FFA level during 24 hours on administration of nicotinic acid in the recommended therapeutic

Table II The diurnal variation of plasma FFA

0 = without treatment Nic = during treatment with nicotinic acid. Somewhat different sampling times were used for cases 1 and 3, 4.

		FFA (mEq/l)									
		Case 1		Case 2				Case 3		Case 4	
Time		0	Nic	0	Nic	Time		0	Nic	0	Nic
7 a.m.		0.54	0.83	0.81	0.47	7 a.m.		0.70	0.70	0.94	1.05
9 a.m.		0.18	0.26	0.39	0.21	10 a.m.		0.33	0.19	0.44	0.32
11 a.m.		0.29	0.40	0.43	0.84	12 a.m.		0.53	0.14	1.09	0.32
1 p.m.		0.19	0.26	0.7	0.33	1.30 p.m.		0.40	0.59	0.73	1.31
3 p.m.		0.10	0.16	0.15	0.17	4 p.m.		0.70	0.22	0.96	0.70
5 p.m.		0.52	2.50	0.83	0.40	5 p.m.		0.83	0.40	1.17	2.05
7 p.m.		0.14	0.30	0.16	0.60	6.30 p.m.		0.45	0.44	0.30	1.45
9 p.m.		0.17	0.68	0.22	0.36	9 p.m.		0.44	0.77	0.26	1.08
12 p.m.		0.76	2.30	0.32	0.27	11.30 p.m.		0.79	0.29	1.08	0.0
2 a.m.		0.63	1.36	0.77	1.96	1.30 a.m.		0.69	1.57	0.86	2.58
4 a.m.		0.56	1.01	—	—	4 a.m.		0.61	0.95	0.78	1.17
7 a.m.		0.54	0.93	0.47	1.10	7 a.m.		0.53	0.93	0.77	1.25

dose. This is not compatible with the hypothesis that the effect of nicotinic acid on the plasma lipids is secondary to an inhibition of FFA mobilization (3). Apparently another explanation must be sought as the plasma triglycerides and cholesterol were depressed in all our patients in spite of an increase in the mean FFA level.

ACKNOWLEDGEMENT

This work was supported by the Swedish Medical Research Council (Project No. B 6713 X 2063-0).

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PRIMARY AMYLOIDOSIS IN LUNGS AND HEART

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Abstract A case of presumably primary amyloidosis, located in lungs and heart in a 70 year-old man is reported. During the last three years of his life the patient presented symptoms of congestive heart disease. The diagnosis was made at post mortem microscopic examination. The frequency of primary amyloidosis of lungs and heart is commented on.

Amyloid deposition in the lungs is reputed to be a rare condition in the primary as well as in the secondary form of amyloidosis. The frequency is estimated somewhat differently by different authors. Spencer (8) in Pathology of the Lung mentions that primary amyloidosis is an unusual finding and secondary amyloidosis very unusual.

Some publications suggest that amyloid deposition in the lungs in the primary generalized form of the disease is not an altogether unusual finding. In fact Rukavina et al (7) from post mortem examinations of 142 cases of primary generalized amyloidosis found the lungs to be moderately to considerably involved in 44 cases (30.9%).

Symmers (9) in a material of 145 cases of primary generalized amyloidosis found infiltration of the lungs in about 30% of the cases.

Weiss (12) mentions that the lungs are involved in nearly half of the cases of primary generalized amyloidosis.

The situation differs in the cases when the amyloid deposition is totally or mainly located in the lungs.

Hambach (2) reports the number of published cases of that kind not to surpass 35.

Prowse (6) could collect only 18 authentic cases of amyloidosis of the lungs.

Tennstedt (11) mentions that compared with other organs amyloid is found only rarely in the lungs; this is especially true of the secondary

form of the disease. In primary amyloidosis the lungs seem to be more frequently attacked.

In Denmark Noring and Paaby (5) have described a case of amyloid deposition in the deep paths of respiration and Sprensen (10) a case of tumor like amyloid deposition in the lung.

In the heart amyloid deposition is seen more frequently than in the lungs. Rukavina et al (7) found the heart to be involved in 92 of 142 cases (64.7%).

Symmers (9) in primary generalized amyloidosis found the heart to be involved in no less than 90% of cases.

Several publications point out a relation between amyloidosis of the heart and high age. Thus Lang (4) reports five cases of primary amyloidosis located in heart and lungs; the average age was 87.8 years.

Josselson et al (3) report 29 cases of primary amyloidosis with the heart as the only affected organ. The average age was 82 years.

In the following a case of presumably primary amyloidosis in lungs and heart is reported. The diagnosis was made at microscopic examination of tissues removed post mortem without anyone previous suspicion of the disease.

For this reason only tissues from a few organs were removed for histological examination and so it may reasonably be said that the examination concerning the extension of the amyloidosis has not been comprehensive enough.

Despite this the case is presented partly because primary amyloidosis, especially in the lungs is remarkable and partly in order to draw attention to the diagnosis of pulmonary amyloidosis as one of the possibilities to be considered when the clinical roentgenological and patho-ana-

tomical findings presented by this patient are encountered

CASE REPORT

A 70-year-old married man a former mechanic was admitted to the Sundby Hospital, Medical Department on December 12, 1963 because of congestive heart disease and degeneration of cervical intervertebral disc

Prior to admission, he had been in good health and never hospitalized but in 1957 he had consulted the medical out patient department of the Finsen Institute. The diagnoses there were neuritis in radiales osteochondrosis articularis unchovertebrales myositis. He was treated with aneurin injections and physiotherapy ESR was then 39 mm h, Hb 97%

He was admitted because of weakness that had grown worse through three years dyspnoea and crural oedemas. Sometimes he had nocturnal fits of dyspnoea. The patient denied having ever had rheumatic fever. There was no information on the consumption of tobacco. Moreover during the last seven years he had been suffering from paraesthesia and gradually developing pains in the three radial fingers of each hand. The sensation in these fingers had decreased and wounds healed slowly. The patient claimed to tolerate all kinds of food.

He had stopped working three years earlier but not because of illness. His own doctor had treated him with digitalis and diuretics.

On admission to Sundby Hospital his general condition was found to be poor nutritional state average respiration slightly quickened there was slight cyanosis of lips and nails. Physical examination of the lungs was normal the heart action was irregular with pulse deficit. No abnormal heart sounds were heard. The liver was palpable about one hand-breadth below the curvature there was no ascites. Massive oedema of both legs. There was muscular atrophy of the thenar muscles of both hands, and the three radial fingers of both hands were tapering, bluish, and weak. All reflexes were normal.

The height was 179 cm, weight 77.5 kg.

The ECG revealed atrial fibrillation and diphasic T waves in all leads.

X-rays of the chest showed considerably enlarged breadth of the heart and increased vascularity of the lungs. There was a questionable exudate in the left pleural cavity. X-rays of the cervical columna revealed osteochondrosis of the 5th (?) cervical vertebra.

Laboratory findings

Hb 149 g/100 ml ESR 40 mm h, fasting blood glucose 74 mg/100 ml urine amylase 150 W units/ml, BP 140/80 the urine was without protein glucose or blood microscopy of urine was normal creatinine 1.3 mg 100 ml creatinine clearance 48 and 64 ml min.

Neurological examination

Bilateral atrophy of the thenar muscles, trophic changes of hands and fingers, a positive Babinski on the left (because of age). X-rays of hands and skull normal EMG of the affected finger muscles revealed neurogenic atrophy. Otological examination because of hoarseness

for some years revealed red and thickened but freely movable vocal chords.

The patient was treated with digitalis and diuretics.

During his stay at hospital the patient did not exhibit any particular symptoms. He was constantly confined to his bed and sometimes he had nocturnal fits of dyspnoea. Temperature and pulse rate were normal.

On January 2 1964 after having been in hospital for about three weeks he suddenly died without any previous increase of temperature or signs of pneumonia.

Post mortem examination

Fibrinous coatings on both pleurae but no exudate. The lungs were large and firm the consistency being nearly liver like. The cut surface was red and homogenous. Small pieces of lung remained floating in water. The bronchial mucosa was haemorrhagic increasingly towards the periphery. There was no secretion in the bronchial tree. The mediastinal lymph nodes were normal. The pericardium was smooth and glistening. There was no liquid in the pericardial cavity. The heart weighed 680 g, measured 13x14 cm i.e. much enlarged. The right ventricle was 5 mm the left ventricle 11 mm. The aortic ostium was narrowed to the size of the fifth finger and in the posterior wall of the left ventricle there were calcifications like those after rheumatic endocarditis. The mitral ostium was unchanged. The myocardium considerably hypertrophic all cavities were dilated. In the posterior wall of the left ventricle there was a 5x6 cm fibrosed area. The coronary arteries and aorta presented severe atherosclerosis. The pulmonary artery and superior caval vein were unchanged.

Oesophagus, ventricle and duodenum were normal.

The liver measured 30x20x8 cm presenting the appearance of a nutmeg liver but the consistency was normal with no evidence of amyloidosis. The spleen weighed 190 g on the cut surface the trabecular pattern was seen to be increased. In the left adrenal gland a yellow brown round tumour was found measuring 2 cm in diameter. There was no evidence of amyloidosis of the adrenals or kidneys.

The skull was not opened. The amyloid test was not done.

Microscopic examination of the lung revealed considerable alterations. In nearly all alveolar walls deposits of an eosinophilic amorphous material were seen, and also in the vascular walls which were nearly diffusely infiltrated. Many vascular walls were narrowed on account of this material which was seen in all layers of the vascular wall (Fig. 1). In a single bronchial branch a solid accumulation of polymorphonuclear leucocytes was seen. A section, stained with methyl violet revealed that this material in some places showed signs of metachromasia. Microscopic diagnosis amyloidosis primaria pulmonum, bronchitis purulenta.

A section from the myocardium disclosed very considerable interstitial fibrosis. Scattered in the interstitial tissue amorphous deposits were seen which were slightly eosinophilic when stained with methyl violet they showed signs of metachromasia. In some areas there were leucocytes, lymphocytes, single plasma cells and polymorphonuclears. Close to areas with the amorphous material



Fig 1 Lung showing amyloid deposits in alveolar and vascular walls HE $\times 125$



Fig 2 Myocardium showing interstitial deposits of amyloid and foreign body giant cell HE $\times 125$

foreign body giant cells were seen (Fig 2). Microscopic diagnosis: amyloidosis primaria myocardii (with foreign body reaction) fibrosis interstitialis myocardii.

In one adrenal gland a cortical adenoma was demonstrated.

The spleen revealed stasis; otherwise no pathological changes.

Microscopy of skin and medulla spinalis did not disclose any pathological changes.

COMMENT

Amyloidosis was diagnosed at microscopic examination of the lungs and myocardium.

As mentioned above the finding was unexpected as during his period of illness the patient had never been suspected of having amyloidosis. At post mortem examination the only grossly remarkable findings were the considerably hypertrophied heart with evidence of earlier endocarditis and the large firm lungs being nearly liver like in consistency. Based upon clinical and post mortem findings the patient was found to

have been suffering from the following recognized diseases:

Endocarditis with later development of aortic stenosis and considerable hypertrophy of the left ventricle. Presumably the patient had had rheumatic fever many years previously though this was denied. However according to the literature there is no basis for supposing any connection between rheumatic fever and amyloidosis.

Osteochondrosis

An about 7 year-old neurologic disease manifesting itself by finger paraesthesiae and neurogenic atrophy of finger muscles.

Purulent bronchitis presumably terminal since the patient had not been particularly annoyed by coughing previously.

None of these diseases may reasonably be suspected of leading to amyloidosis.

Did the patient have multiple myeloma? Unfortunately no examination was made of the serum proteins or of the bone marrow since the diagnosis of multiple myeloma was not suspected clinically. For the same reason no post mortem bone marrow examination was done.

The localization of amyloid in lungs (and myocardium) corresponds with that expected in the primary form of amyloidosis as mentioned above. Secondary amyloid deposition in the lungs is said to be very rare although it may be found.

In agreement with this is the lack of amyloid deposition in the organs generally involved in secondary amyloidosis: kidney, liver, spleen, adrenal gland. Indeed no histological examination was made of kidney and liver but at gross examination these organs were not suspected of amyloidosis.

On the other hand there is no evidence suggesting the presence of any amyloid deposition in the tongue which would have been a very characteristic feature in primary amyloidosis.

The histological finding of giant cells in the myocardium points to primary amyloidosis as this is a rare finding in the secondary form (11).

Moreover it must be mentioned that atypical or even negative staining reactions as in this case are more common in primary than in secondary amyloidosis.

When comparing symptoms with post mortem findings the increasing cardiac insufficiency is found to be in agreement with the considerably enlarged heart, the left ventricle of which especially because of the aortic stenosis was hypertrophic. The severe interstitial fibrosis and the amyloid deposits have further contributed to the cardiac insufficiency.

The patient's dyspnoea was probably due to stasis as well as to the extensive amyloid deposits in the vascular walls of pulmonary vessels and alveolar walls.

The hypertrophy of the right ventricle must probably be put down to the pulmonary changes.

For the clinicians it was natural to interpret the condition as an ordinary although severe cardiac insufficiency and none of the patient's symptoms pointed to amyloidosis nor did the chest X-ray pictures which revealed increased vascular patterns, as in stasis.

Compared with earlier published cases we have found this case to be most like that of Ferris (1).

In his 52-year-old patient the clinical course was characterized by several years of dyspnoea and chronic bronchitis. Post mortem examination revealed nodular amyloid deposits in the vascular walls of pulmonary vessels in alveolar walls and in the bronchioles. In the heart the myofibrils were found to be surrounded by amyloid which again was found in the vascular walls.

In addition our case is much like that of Hambach (2) whose post mortem examination of a 87-year-old woman who for years had been suffering from a moderate dyspnoea revealed amyloid deposition in the lungs partly diffusely located in vascular walls partly tumour-like. Moreover small quantities of amyloid were present in myocardium, spleen and liver.

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DEATH FROM ARTERIOSCLEROTIC HEART DISEASE OUTSIDE HOSPITALS

A Study of 2678 Cases in Stockholm with Particular Reference to Sudden Deaths

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Abstract During the years 1955 1960 1964 1965 and 1966 678 deaths outside the hospitals in the Stockholm region due to arteriosclerotic heart disease (ASHD) were certified by autopsy at the Department of Forensic Medicine Karolinska Institutet, Stockholm. Of these 113 deaths were considered sudden. Owing to detailed police record and autopsy protocols the place and activity at time of death as well as postmortem findings could be studied. In the males below the age of 70 death at work was found to occur less frequently than expected as calculated from the estimated time spent there. Heavy physical exercise and mental stress together were found to precede no more than 5 per cent of the male sudden deaths and only a minority of the female. Autopsy revealed a recent myocardial infarction in 34% of the males below the age of 50 and in about 20% of the older males. Even lower figures were recorded in the female. Possible causes of these apparently infrequent findings are discussed. In agreement with the observations of other authors an overrepresentation of myocardial ruptures was found in the females. Previous symptoms suggestive of ASHD were considered absent in 37% of the male sudden deaths below the age of 50 and in about 0% of the older subjects of both sexes.

There is cumulating evidence of a high mortality within the first hours after the onset of an acute coronary event. Consequently a high proportion of early deaths from arteriosclerotic heart disease (ASHD) occur outside the hospitals and are medically unattended.

The exact characteristics of deaths from ASHD outside the hospitals in a given community such as sudden deaths have not been possible to record. Autopsies carried out by Medical Examiners or Coroners provide some data on deaths outside the hospitals ascribed to ASHD but studies based on their files have been limited primarily to demographic characteristics that have been noted on the death certificate (6).

In view of recent advances in cardiac resuscitation and treatment of serious arrhythmias available data on deaths from ASHD outside the hospitals may serve as an orientation when planning measures for reducing the death toll of ASHD. The present study deals with autopsied deaths from ASHD which have been admitted to the Department of Forensic Medicine Karolinska Institutet Stockholm during five years 1955 1960 1964 1965 and 1966.

MATERIAL AND DEFINITIONS

During the years 1955 1960 1964 1965 and 1966 a total of 678 deaths (1819 males and 859 females) ascribed to ASHD (WHO list diagnosis 470) were autopsied at the Department of Forensic Medicine Karolinska Institutet, Stockholm. Of the ASHD deaths at least 99 had occurred in the Stockholm region. Information was gathered from autopsy protocols and police records the latter containing details such as time and place of death activity at onset and prior symptomatology and medical care. These facts were obtained from testimonies and police investigations made routinely in every case.

The term "sudden death" was used when the following criteria were met: death during mental or physical activity other than rest, where time had not allowed the victim to be brought under medical care. Sudden death at rest was found difficult to evaluate and consequently this category of deaths was excluded from the study.

"Moderate physical or mental activity" included activities like walking, household work and reading, etc.

"Heavy physical exercise" was used to describe activities such as running, walking upstairs and such like.

The term "mental stress" was applied to circumstances such as quarrel, gambling, etc.

No previous symptoms of ASHD were considered to have been present in cases where it had been stated that the patient had not been under medical care for heart disease and no mention of symptoms suggestive of ASHD prior to death had been made.

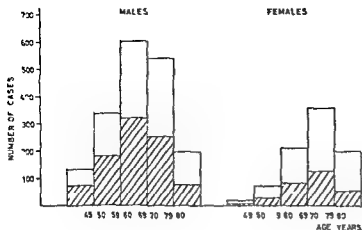


Fig 1 Age and sex distribution of deaths from ASHD outside the hospitals (////) sudden deaths — not sudden deaths

RESULTS

According to the above mentioned criteria sudden death occurred in 1213 cases (912 males and 301 females) out of the total of 2678 deaths (1819 males and 859 females). The overall male/female ratio was 2.1:1 as compared to 3:1 for the sudden deaths.

The mean age in the whole series was 65.7 years for males and for females 71.2 years; a difference of 5.5 years. In the group of sudden deaths the age difference was 4.4 years; the mean age of the males and females being 65.0 and 69.4 years respectively. Age and sex distributions of the deaths are shown in Fig 1.

Activity preceding sudden death is given in Table I. Following the above definition deaths at rest were excluded from the sudden deaths. It is seen that among males heavy physical exercise

and mental stress together preceded 26% of the deaths below the age of 50 and close to 16% in the higher age groups. However, in the female sudden deaths these activities were noticed only in a negligible minority.

From Fig 2 it is seen that in all age groups the male sudden deaths occurred nearly as frequently outside home (work included) as at home. A somewhat smaller proportion of the females died outside their homes.

As seen in Fig 3, recent myocardial infarction was diagnosed at autopsy in 34% of male sudden deaths below the age of 50. The corresponding female age group was too small to permit comparison. From the age of 50 and on, recent myocardial infarction was found in approximately 20% in both sexes.

A myocardial infarction more than one week old was found in 32% of the males below the age of 50. The corresponding figure for the higher

Table I Activity preceding sudden witnessed death (death at rest excluded according to definition)

Age	Moderate physical or mental activity	Heavy physical exercise	Mental stress	Total
Males				
<49	54 (74%)	18 (75%)	1	73 (100%)
50-59	154 (84%)	75 (14%)	5 (7%)	184 (100%)
60-69	267 (83%)	45 (14%)	11 (3%)	323 (100%)
70+	290 (88%)	31 (9%)	11 (3%)	332 (100%)
Females				
<49	8	0	0	8
50-59	29	0	0	29
60-69	81	0	1	84
70+	175	2	3	180

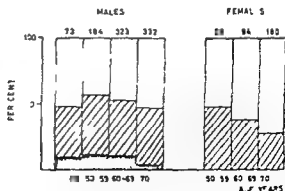


Fig 2 Place of sudden deaths. Females <49 years not included (n=8). (////) death at work (except home) — death at home

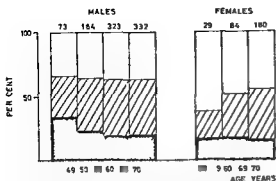


Fig 3 Autopsy findings in sudden deaths ascribed to ASHD. Females -49 years not included ($n=8$) \blacksquare recent myocardial infarction (< one week old) \hatched myocardial infarction > one week old \square ASHD without reference to thrombotic occlusion

age groups was 43%, with a narrow range. In the females below the age of 60 myocardial infarction more than one week old was seen in 21%. The older females were found to have an old myocardial infarction nearly as frequently as the males of corresponding age (Fig 3).

The average proportion of sudden deaths from ASHD without reference to thrombotic occlusion was found to be somewhat higher in females than in males (Fig 3).

The distribution of myocardial ruptures is shown in Table II. The overall proportion of sudden deaths due to myocardial rupture in males and females was 3.2 and 4.7% respectively. An increased rate of myocardial ruptures with age was seen in both sexes. In all cases of myocardial rupture a recent myocardial infarction was diagnosed and hence classified as recent myocardial infarction in Fig 3.

Previous symptoms of ASHD were considered absent in 249 (191 males and 58 females) out of 1213 sudden deaths (20%). A presentation of these deaths is given in Fig 4. Sudden death as first manifestation of ASHD was recorded in 37% of the males below the age of 50. In the

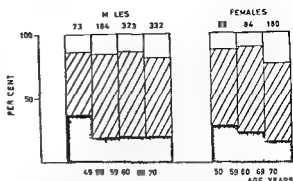


Fig 4 Sudden death and previous symptoms suggestive of ASHD. Females -49 years not included ($n=8$) \blacksquare no previous symptoms suggestive of ASHD \hatched previous symptoms of ASHD \square uncertain

older males the corresponding figure was close to 20%.

Although there was only a small number of women in the younger age groups a similar tendency towards a higher percentage of sudden death as first manifestation of ASHD was found in the females.

Autopsy findings in subjects without previous symptoms of ASHD are shown in Fig 5. Recent myocardial infarction was found in 63% of the males below the age of 50. Beyond this age a finding of recent myocardial infarction was made in about 25% of both sexes. A myocardial infarction more than one week old was found in approximately 20% of the males unrelated to age. ASHD without thrombotic occlusion was found in 19% of the males below the age of 50 and

Table II Myocardial ruptures in sudden deaths

Age	49-59	60-69	70-	Total
Males				
0/54	1/184 (1)	12/323 (4)	15/33 (5)	29/917 (3.2)
Females				
0/8	0/29	2/84 ()	1/180 (7)	14/301 (4.7)

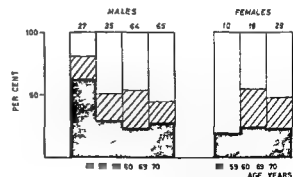


Fig 5 Autopsy findings in sudden deaths without previous symptoms suggestive of ASHD \blacksquare recent myocardial infarction (< one week old) \hatched myocardial infarction > one week old \square ASHD without reference to thrombotic occlusion

in the higher age groups this was seen in approximately 50%. In the females the autopsy finding were essentially the same

DISCUSSION

Like other studies on deaths from ASHD outside the hospitals based on the files of Medical Examiners and Coroners the present one represents a selected material. An unknown number of such deaths in the Stockholm region was not referred to the Department of Forensic Medicine. In the Stockholm region the Department of Forensic Medicine in addition to carrying out strictly forensic investigations also has a function similar to that of the Coroner or Medical Examiner performing autopsies in cases of non violent death when a doctor sufficiently familiar with the patient to be able to issue a death certificate is not available. Of all cases brought to the Department during the time studied autopsy was carried out in 85%. Autopsy was not carried out in cases when a physician was willing to issue a death certificate provided that the police had no objection to this procedure. It was thought that the figures given in the present study could be regarded as an estimated minimum of sudden deaths from ASHD outside the hospitals in the Stockholm region a city community of about one million inhabitants during the years studied.

The above mentioned criteria of suddenness were set arbitrarily. No uniform definition has as yet appeared in other studies as has been pointed out by Kuller (6) in his extensive review of the literature on this subject.

The largest number of sudden deaths was recorded in the higher age groups in both sexes the mean age of the males and females was 65.0 and 69.4 years respectively. The average age of death from ASHD at a general hospital in Stockholm like the Serafimer hospital during the period studied was 68.0 (males) and 72.0 years (females) a slight difference to which no significance can be attributed owing to the above mentioned limitations of the study. However the percentage of sudden deaths according to the criteria used in the present study was slightly higher in the lower age groups of both sexes (Fig. 1). According to the definition of sudden death almost all cases of sudden death were witnessed the lower percentage of sudden deaths

in the old females could thus be a consequence of a higher proportion of women living alone due to the longer life span of the females.

In the literature there is little information about circumstances associated with sudden deaths. In the present study detailed police records made reconstructions possible. It may seem remarkable that so few deaths occurred in association with physical exercise and mental stress. Further confirmation on this point is needed.

On account of the low coincidence of heavy physical exercise and mental stress with sudden death it was not surprising to find so few deaths occurring at work. Because of possible medico-legal implications it is assumed that a relatively higher proportion of the deaths at work was referred to the Department of Forensic Medicine and that autopsy was carried out in every case. This in turn could lead to an overrepresentation of sudden deaths occurring at work. Assuming that 40 hours per week were spent at work and that vacation and public holidays together accounted for one month per year the time spent at work would represent about 20% of the year. In the present study no more than 12% of the male sudden deaths below the age of 70 occurred at work. Kuller *et al.* (7) in an epidemiological study on sudden deaths between 40 and 64 years found that 15% of the deaths occurred at work where it was estimated that 25 to 30% of the time was spent.

Postmortem findings should be considered in relation to the sudden character of death. Myocardial infarction cannot be demonstrated by routine technique as used in this study until several hours after its emergence (2). However examining serial sections and the microscopic appearance Baroldi (1) could demonstrate no acute or recent coronary occlusion in 54% of 125 sudden but not unexpected "coronary heart deaths". A normal myocardium was found in 31% of these cases. Coeval myocardial and coronary lesions were demonstrated in only 19% of the 125 cases. In a very detailed study including postmortem angiography and microscopic dissection Crawford (3) found recent thrombotic occlusion in 41% of 100 sudden deaths ascribed to ASHD. The results of these studies may serve as an explanation for the relatively frequent finding of ASHD without reference to thrombotic coronary occlusion in the present study.

Myocardial rupture as a cause of sudden death was found to be increasingly frequent with age. This finding is supported by two British studies (4-8). Mitchell and Parish (8) in a study of 36 cases of myocardial rupture found that half of these occurred within 24 hours after the onset of symptoms. The same authors found a female preponderance, the male:female ratio being 1:1.2. Crawford and Morris (4) reported a male:female ratio of 1:0.6 under the age of 70. Above this age the ratio was reversed to 1:1.7. Both studies were based on hospitalized and Coroner cases combined. In two Swedish studies on hospitalized patients Sievers et al. (9) found a male:female ratio of myocardial ruptures of 1:1.2. In a later study Sievers (10) reported a male:female ratio of 1:1.4. Agreeing with these results the male:female ratio in the present study was found to be 1:1.5.

It is rather interesting that in males above the age of 50 the proportion of cases in which sudden death was the initial manifestation of ASHD was close to 20% of all sudden deaths from ASHD. In the youngest male group sudden death as first manifestation of ASHD was found in 37% of the sudden deaths, a not unexpected difference. Kannel et al. (5) in a report from the Framingham study found that sudden death was the first manifestation of ASHD in 20% of all new cases of ASHD exclusive of angina pectoris. The results of this study indicate that even in a community with a carefully planned search for signs of ASHD a substantial proportion of sudden deaths occurs before any clinical signs of ASHD have appeared.

Recent myocardial infarction might be expected to be a more common finding in sudden death not preceded by any symptoms of ASHD because of a more careful search for the cause of death. Baroldi (1) found a normal myocardium as judged from serial sections and microscopic examination in 53% of 116 sudden coronary deaths without previous symptoms of ASHD. A recent thrombotic occlusion has likewise been found in 53%. However coeval thrombotic occlusion and myocardial lesion were found in only 22%. It is rather puzzling that such a high proportion of fatal events seems to lack anatomical evidence.

The total annual numbers of sudden deaths from ASHD outside the Stockholm hospitals dur-

ing the time studied were not possible to record. However the rough figure of 300 cases recorded in the present study during each of the last three years (1964-1965 and 1966) could be regarded as a minimum number of sudden deaths from ASHD outside the hospitals in the Stockholm region, a city community of about one million inhabitants. In the light of the present techniques of resuscitation and management of serious arrhythmias this largely overlooked category of patients now dying medically unattended stands out as one of the most urgent problems of coronary heart disease to-day.

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IN VITRO MIGRATION OF PERIPHERAL HUMAN LEUCOCYTES IN CELLULAR HYPERSENSITIVITY

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Abstract The influence of various antigen concentrations upon the in vitro migration of peripheral leucocytes from persons with cellular hypersensitivity to *B abortus* Bang is investigated. An inhibition as well as a stimulation of the cell migration can be demonstrated. The dual effect of the antigen upon the migration cultures seems to be dependent upon two factors: first the antigen concentration, and second the sensitivity of the cell donor. High antigen concentrations lead in all cases to inhibition of the cell migration. Low antigen concentrations tend to result in a stimulation of the cell migration if the cells are of a low to moderate degree of sensitivity while cells of high sensitivity are still inhibited in the low concentrations.

According to the generally accepted view the action of antigen in vitro upon immunocompetent cells results in an inhibition of the cell migration if the cells originate from an organism in a state of cellular hypersensitivity to the same antigen. This observation has been confirmed in many animal experiments (2, 3, 4, 6, 9, 10, 12, 13, 14) and recently in studies in man (18, 19). The inhibition of the cell migration corresponds well to the sensitivity of the cell donor as expressed by the delayed intracutaneous reaction in a way that a more pronounced inhibition of the migration is paralleled by a more intense intracutaneous reaction. There have, however, been a few reports indicating that under special experimental circumstances a stimulation of the cell migration may be seen as well (7, 11, 15, 16).

Juhász-Schaffer, using fragments from the spleen and the kidney, was able to show a stimulation of the cell migration in the presence of tuberculin in rather low concentrations if the cells originated from tuberculin sensitized animals. Hall and Scherago, in experiments with peripheral human leucocytes, showed that tuberculin sometimes caused an inhibition, sometimes a

stimulation and finally in some cases no alteration of the cell migration could be observed. The authors considered the results contradictory and concluded that the method in their hands was not able to demonstrate cellular hypersensitivity in vitro.

Švejcar and Johanovsky (15) in their study with spleen fragments demonstrated that cells of tuberculin sensitive guinea pigs in the presence of specific antigen showed an increased migration activity in the first hours of incubation compared with the control cultures. Later in the experiments the usual inhibitory effect of tuberculin was observed so that the final result after 24 hours was an inhibition of the cell migration.

Švejcar and coworkers (16) were also able to demonstrate that in some cases the cells were permanently stimulated if low antigen concentrations were applied.

It thus seems suggestive that there is a relationship between the antigen induced inhibition and stimulation of the migration of immunocompetent cells in vitro and that both types of reaction may be expressions of cellular hypersensitivity.

The purpose of the present work is to study further this interrelationship and to analyze some of the factors involved in the action of the antigen upon the cell migration. *Brucella* hypersensitivity is used as an experimental model because the antigen induced inhibition of migration of peripheral human leucocytes in vitro has proved to be a specific parameter of cellular hypersensitivity (18).

MATERIAL AND METHODS

The material consisted of 97 *brucella* positive and 82 *brucella* negative persons.

Table I The migration indices of brucella negative and brucella positive persons at various antigen concentrations

N = number of observations \bar{X} = mean value S = standard deviation P = statistical difference of the mean value in relation to the brucella negative observations.

Antigen conc (bact/ml)	Brucella neg.				Brucella pos.															
	N	\bar{X}	S		N	\bar{X}	S	P	N	\bar{X}	S	P	N	\bar{X}	S	P	N	\bar{X}	S	P
50 mill	82	0.90	0.07	.74	0.73	0.02	<0.001		59	0.65	0.03	<0.001	48	0.55	0.03	<0.001	16	0.4	0.09	<0.001
25 mill	33	0.93	0.08	.8	0.84	0.10	<0.05		0	0.74	0.1	<0.01	20	0.68	0.07	<0.001	8	0.58	0.07	<0.001
10 mill	53	0.96	0.08	.19	1.11	0.17	<0.01		35	0.91	0.15	>0.05	31	0.77	0.10	<0.001	10	0.65	0.11	<0.001
5 mill	10	0.99	0.08	.10	1.15	0.17	<0.02		19	1.02	0.17	>0.05	13	0.80	0.11	<0.001	—	—	—	—
2.5 mill	22	0.98	0.06	.7	1.23	0.13	<0.01		6	1.36	0.23	<0.02	—	—	—	—	—	—	—	—
1 mill	21	1.01	0.11	.11	1.08	0.21	>0.05		16	1.21	0.27	<0.01	20	0.99	0.28	>0.05	10	0.73	0.16	<0.01

Brucella hypersensitivity was defined in the following way

A migration index below 0.78 at an antigen concentration of 50 mill brucella bact per ml (see below) which has been shown to be the borderline between the brucella positive and brucella negative observations (18)

Persons were considered brucella negative if they did not fulfil the above mentioned criterion

The hypersensitive group consisted of 37 spontaneous brucella positive persons and of 65 persons examined after the injection of 1000 mill killed brucella bact (*Brucella abortus* Bang). Some of these cases were studied repeatedly after the vaccination

Leucocyte migration studies

Migration studies were performed with peripheral human blood leucocytes according to the technique initiated by Jendryen and described in detail in a previous paper (17)

The action of the antigen upon the cell migration was expressed in the migration index M/M where M represents the average migration area of the antigen containing cultures and M the average migration area of the control cultures. Thus the numerical value of the migration index expresses whether the migration has been stimulated or inhibited by the antigen. An index exceeding 1.00 indicates stimulation and an index less than 1.00 inhibition of the cell migration

In each experiment the migration index was determined at a high concentration of brucella antigen, i.e. 50 mill bact per ml. Furthermore the migration indices were determined at one or more of the following concentrations of brucella antigen: 25 mill, 10 mill, 5 mill, 2.5 mill and 1 mill bact per ml. The cells in each experiment were examined at least two different antigen concentrations. Because of the rather limited amount of cultures which could be obtained it was not possible in each experiment to determine the migration indices at all the different antigen levels

RESULTS

Table I shows the distribution of the migration indices at the different antigen concentrations

The brucella positive observations have been divided into four groups according to their degree of sensitivity i.e. the migration indices at an antigen concentration of 50 mill bact per ml. The least sensitive group consists of all observations with migration indices from 0.71–0.76 the next two groups comprise values from 0.61–0.70 and 0.51–0.60 and the most sensitive group represents migration indices below 0.50. This separation is of course to some extent arbitrary but builds upon the fact that the migration indices at 50 mill bact per ml have been shown to be well correlated to the sensitivity of the cell donor as expressed by the intracutaneous reaction (18)

It appears (Table I) that at the low antigen concentrations there is a tendency to stimulation of the migration as indicated by migration indices over 1.00

On the other hand the migration indices from the brucella negative group do not show this alteration. The values are all placed around 1.00 with minor variations at the different antigen concentrations

The mean values of migration indices in brucella positive cultures at the different antigen levels are significantly different from the mean values of migration indices in brucella negative cultures at nearly all levels with a few exceptions. In these cases however the standard deviations are significantly different

In order more clearly to illustrate the relationship between the concentration of antigen and its influence upon the cell migration the mean values have been plotted in a diagram (Fig. 1). At the high antigen concentration the migration indices from the brucella positive persons are all well

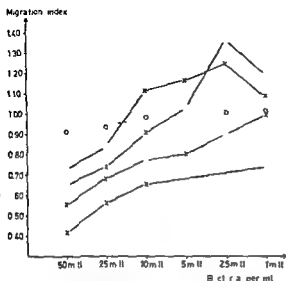


Fig 1 The migration indices at various antigen concentrations x—x brucella positive o—o brucella negative

below 1.00. With decreasing antigen concentrations in the least sensitive groups first a disappearance of the migration inhibition is seen followed by a stimulation whereas the migration of the highly sensitive group remains inhibited at the lowest concentrations although to a smaller degree. The curve thus seems to be biphasic starting with low indices followed by an interval with indices within the normal range continuing with high indices and finally going down into the normal range. It also appears from the diagram that at a fixed low antigen concentration a wide distribution of indices is observed indicating that in some cases the migration has been stimulated in some inhibited and in some left uninfluenced by the antigen.

DISCUSSION

The data presented indicate that the action of antigen upon sensitized cells may influence the migration in apparently two ways: inhibition or stimulation. The final result seems to be dependent upon at least two factors: 1. the antigen concentration and 2. the sensitivity of the cells. At a fixed sensitivity the outcome will depend upon the antigen concentration in such a way that high concentrations induce an inhibition and low concentrations a stimulation. If on the other

hand the antigen concentration is fixed at a relatively low level the outcome will depend upon the sensitivity of the cells: a highly sensitive culture being inhibited and a less sensitive culture stimulated or perhaps apparently uninfluenced.

Inhibition and stimulation may thus both be considered expressions of cellular hypersensitivity *in vitro* and seem to be closely interdependent.

The above mentioned experiments by Juhász-Schaffer (11), Hall and Scherago (7) and Švejcár and Johanovsky (16) may support this theory. Juhász-Schaffer, using spleen and kidney fragments from guinea pigs, was able to show only stimulation after addition of tuberculin antigen. The antigen concentration was however low, namely a 1:3000 dilution of OT, whereas in a similar system where inhibition was a constantly occurring phenomenon the concentration was 1:100 of OT (14). The two experiments cannot be directly compared: first of all because the degree of sensitivity of guinea pigs must be different due to the various immunization procedures applied. Nevertheless it seems reasonable to conclude that the stimulation seen in Juhász-Schaffer's experiments at the low antigen concentration is related to the inhibition seen in Švejcár and Johanovsky's experiments at the high antigen concentration in the same way as described in the brucella system. Švejcár and Johanovsky in their later experiments (16) have more directly established this proposed connection. They investigated the migration of sensitized spleen cells from guinea pigs at various antigen concentrations and found that at the lower concentrations there was a tendency to stimulation of migration while the migration was always inhibited at the high concentrations. The stimulation observed was statistically significant at the 5% level at two of the low concentrations but otherwise the picture was a wide distribution of indices at the various antigen levels. This might be expected because no attempt was made to divide the material according to the sensitivity of the cell donors. In other words, only one of the factors which determine the final outcome of the cell migration was analyzed, namely the antigen concentration.

Conversely Hall and Scherago in their experiments with human leucocytes tried to separate the patients somewhat according to the degree of sensitivity, i.e. the severity of the tuberculous infection. The experiments were as a whole not

very successful because the authors were not able to show a constantly occurring tuberculin induced inhibition of migration not even in the most sensitive group. This negative result may be explained by the fact that the antigen concentration applied was too low. Indices from the patients belonging to the least hypersensitive groups in some cases showed values exceeding 100 indicating a stimulation of the migration which was never observed in the highly sensitive groups. The deviations were not statistically significant and the main impression was again a wide distribution of indices. These experiments probably represent an analysis of the other factor involved namely the sensitivity of the cell donor.

In the present experiments the antigen concentration as well as the sensitivity of the cell donor has been considered and this double aspect seems to give a clearer picture of the relationship between stimulation and inhibition of the cell migration. Apart from the theoretical importance of this relationship some facts of practical value in the detection of cellular hypersensitivity *in vitro* can be extracted. In the first place a stimulation of migration may be considered an expression of cellular hypersensitivity just as specific as an inhibition of migration. Secondly it is necessary to use very high, i.e. subtoxic antigen concentrations to ensure an inhibition of migration in all cases examined. If too low antigen concentrations are used the picture will be confusing with stimulation, inhibition or no changes of the migration at all, and it will be impossible to correlate the findings with the intracutaneous reaction. It should also be clear that the degree of stimulation determined at a single antigen concentration cannot be used as a parameter of cellular hypersensitivity because it is impossible to predict at which point on the biphasic curve the value is recorded in other words whether it is placed on the ascending part, the top or the descending part.

If the experimental circumstances do not allow the application of an antigen concentration as high as desirable there still exists the possibility to determine the sensitivity of the cell donor by using falling concentrations of antigen. Determination of migration indices at various antigen concentrations will permit at least part of the biphasic curve to be established thereby providing a more exact expression of the degree of

sensitivity than a determination of the migration index at a single antigen concentration.

The mechanism of the stimulation of migration is just as obscure as the mechanism of inhibition, but it seems reasonable to assume that both are due to the same fundamental biologic process. In animal experiments it has been shown that the inhibition is dependent upon a substance excreted by the sensitive lymphocytes upon contact with the antigen (1, 5, 8). Preliminary experiments have shown that a similar factor may be instrumental in the *in vitro* system used in the present study.

The effect upon the migration might simply be related to the resulting concentration of active substance in the tissue culture medium: a high concentration resulting in inhibition and a low concentration in stimulation. Investigations to prove this hypothesis are in progress. The experiments by Svejcar and Johanovsky (15) indirectly support this theory. As earlier mentioned, they determined the migration indices at different hours after the addition of antigen and observed that in the beginning there was a stimulation which later disappeared and was gradually converted into an inhibition of the cell migration. The most likely explanation seems to be that the concentration of the active substance is at first low resulting in stimulation and later as the production continues and the concentration rises the cells will be inhibited in their migration.

The interaction between sensitivity and antigen concentration as visualized by the biphasic curve thus seems to be an expression of a general biologic phenomenon in which small stimuli result in stimulation or proliferation while an increase of the stimuli leads to inhibition or destruction.

ACKNOWLEDGEMENTS

The present study was supported by Fonden til lægevidenskabens fremme and Den lægevidenskabelige forskningsfond for Storkøbenhavn, Færøerne og Grønland.

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PERCUTANEOUS RENAL BIOPSY ON URAEMIC PATIENTS AIDED BY SELECTIVE ARTERIAL ANGIOGRAPHY AND ROENTGEN TELEVISION

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Abstract Selective renal arterial angiography and television monitored fluoroscopy have been used in locating the kidney at percutaneous renal biopsy in 68 patients including 40 with uraemia. This procedure makes the renal arteries and the contour of the kidney clearly visible. The tip of the biopsy needle can be placed at a suitable point on the kidney surface away from the arteries and the risk of bleeding seems thus to be reduced. No serious clinical complications occurred. So far no toxic damage to the renal tissue has been noticed referable to the contrast medium. Renal angiography is preferable as a means of localizing the kidney when needle biopsy is to be performed in cases of chronic renal failure in obese and heavily built patients and in cases of nephrosis and overhydration. In these cases the method merits routine use. It is not necessary to perform renal arterial angiography as an aid to renal biopsy in patients with normal or moderately impaired renal function if the kidneys can be visualized at fluoroscopy directly or after urography. In acute renal failure we think that retrograde pyelography can be used but it is not the preferable method.

Television monitored fluoroscopy has proved to be a useful aid in the procedure of percutaneous renal biopsy (3, 4, 6). A prerequisite is that the contour of the kidney is sufficiently clearly definable on the television screen.

One can only occasionally see the kidneys directly at fluoroscopy. In patients with normal renal function intravenous administration of a contrast medium which accumulates in the renal parenchyma (urography) will markedly increase the visibility of the kidneys. Large kidneys of severely uraemic patients (creatinine exceeding 6 mg per 100 ml of serum) are occasionally displayed directly if the patients are effectively dehydrated and adequately purged and if intestinal gas is eliminated (6). In these cases however

urography will never improve the visibility of the kidneys. The contours of small kidneys are according to our experience never visible at television monitored fluoroscopy if the patient has advanced uraemia even if an intravenously administered contrast medium is given in large and repeated doses and for several (up to 24) hours before the biopsy.

We have performed renal biopsy on uraemic patients after the renal pelvis has been filled with a contrast medium by retrograde pyelography on the side where the biopsy was done (7). In that paper we have reported attempts at renal biopsy in connection with renal arterial angiography. Experience has now been gained concerning the value of this procedure.

Selective renal arterial angiography has been performed bilaterally on all the patients as part of the investigation of their disease and to exclude malformations or morphological changes that would make renal biopsy dangerous to the patient (arterial aneurysms, unilateral aplasia or advanced unilateral hypoplasia of the kidney). The needle biopsy was usually done immediately after the angiography. In a few cases angiography and biopsy could not be combined. A catheter was then inserted into the renal artery only to allow renal biopsy to be carried out.

TECHNIQUE

The patients are purged with castor oil on the day before the biopsy and should not be given any fluid for the last 12 hours. A water enema (1, 2) is given two hours before the biopsy is to be done. One and a half hours before the biopsy water-soluble vasopressin (Postacton®) is injected subcutaneously. No malweight



Fig. 1 Roentgen film exposure taken during injection of contrast medium into the renal artery showing the biopsy instrument introduced into the right kidney of a patient weighing 63 kg. His creatinine was 8.7 mg per 100 ml of serum. The kidney measured 10.5×5.4 cm and the renal cortex 3–4 mm in thickness on the roentgen film. The biopsy yielded 11 mm of renal tissue which showed chronic glomerulonephritis on microscopical examination. No complications were noted.

patients receive 10 IU heavy patients (more than 80 kg) 15 IU and light patients (less than 55 kg) and children 5 IU. The patients are premedicated with morphine scopolamine according to weight and age.

The position of the first and second lumbar vertebrae and the tip of the twelfth rib are marked with a lead shot, which is fastened with adhesive plaster on the skin of the back of the patient. After local anaesthesia a red Odman-Ledin catheter (AB Lafa, Stockholm) is inserted by the Seldinger technique into the femoral artery through the groin. The procedure is controlled by fluoroscopy using an image intensifier combined with television. Selective renal diagnostic angiography is then performed bilaterally (1–8) after which the catheter is left with its tip lying in the abdominal aorta. The roentgen films are examined and it is decided on which side the biopsy is to be done. The bent tip of the catheter is placed in the renal artery on the side where the biopsy is planned and the patient is then carefully turned over into the prone position. He should lie with his legs stretched and not co-operate in order to lessen the risk of the tip of the catheter slipping out of position. After the patient has been turned, a check is made by fluoroscopy to make sure that the tip of the catheter is still in the renal artery.

When 1–2 ml of the contrast medium (60 Urografin®) is injected into the renal artery catheter the oper-

ator can watch on the television screen the contrast medium passing rapidly through the large arteries and accumulating in the parenchyma rendering the kidney visible for a minute or two. The track down to the surface of the kidney is infiltrated with a local anaesthetic and by measuring the length of the free end of the needle one can calculate how deeply the tip of the needle is situated. The position of the tip of the anaesthetic needle on the renal contour is checked by fluoroscopy and changed if necessary (7). A Franklin Vim Silverman needle 12 cm long and sharply ground is then introduced the same way and to the same depth as the anaesthetic needle. The resistance offered by the renal parenchyma and the movements of the instrument with the respiration and arterial pulse wave of the patient indicate that the kidney has been reached. Contrast medium is injected into the renal artery catheter and the operator checks by fluoroscopy that the tip of the biopsy instrument is situated in the lower pole of the kidney at a safe distance from the large arteries (Fig. 1). The patient is told to hold his breath and the biopsy is done with vigorous rapid movements. If no renal tissue or too short a piece is extracted at the first attempt the biopsy should be repeated at most twice and the position of the needle should be checked before each attempt. The biopsy tissue is arranged in spiral form on a dry slide and fluid agar agar is poured over it. By examination under the dissection microscope it can be ascertained that the tissue contains glomeruli (5).

The patient is confined to bed for observation until the following day.

RESULTS

Renal biopsy by the technique described has been performed on 68 patients. Thirty-four of these had severe uraemia (creatinine 6–15 mg per 100 ml of serum) and sixteen slight uraemia. Renal function was normal or slightly impaired in 18 patients. The pieces of tissue retrieved at biopsy were sufficient for diagnosis in 57 cases. We have found the length and sharpness of the biopsy instrument to be of great importance for the outcome of the biopsy attempts (7).

Complications referable to the arterial punctures were no more frequent in our cases than in those described by others (1). We could not find that any complications were caused by the tip of the catheter being left in the renal artery for a relatively long time. The amounts of contrast medium given during fluoroscopy in connection with the biopsy procedure (totally 3–12 ml) are the same as those administered during diagnostic selective renal angiography. The repeated injections of contrast medium do not seem to give rise to any clinically demonstrable disadvantages. The uraemic state of the patients was unchanged af-

ter the biopsy or increased at the same rate as before the procedure. Our results accord with those of other investigators who have found that an impairment of the renal function cannot be demonstrated in healthy persons after renal angiography (2).

Histological examination under the light microscope has so far not shown any evidence of acute toxic effects in the retrieved pieces of tissue. In the biopsy specimens from the patients with uraemia the histopathological changes caused by the disease were so advanced that it could not be decided whether further acute damage had been caused. In the material there are three patients with proteinuria but no other evidence of renal disease. The biopsy specimens showed histologically normal renal tissue in these cases.

No serious clinical complications were seen apart from transient shock immediately after the biopsy in one patient with amyloidosis. There were no signs of local bleeding. It is not clear whether the shock was due to blood loss, pain or hypersensitivity to the contrast medium. The patient had reacted in the same way at a previous examination when he was given a contrast medium intravenously. One patient had gross haematuria for three and another intermittent haematuria for 14 days.

We have not performed renal arterial angiography after the biopsies and the material has not been investigated with regard to late complications. The possibility of intrarenal damage caused by the biopsy attempts e.g. arteriovenous fistulas cannot be excluded. So far however we have not noticed any clinical symptoms indicative of late complications.

COMMENTS

The method described enables the clinician to carry out renal biopsy in uraemic patients even if their kidneys are small and renal function is greatly impaired. It allows repeated and careful checking of the position of the biopsy needle in relation to the contour of the kidney and the large arteries.

The methods of displaying the kidneys at renal biopsy should be chosen with regard to the body build of the patient, the nature of the renal disease and the degree of impairment of renal function.

In patients with normal renal function the kidneys can as a rule be seen directly at fluoroscopy after administration of a contrast medium intravenously. If the patient is oedematous, very fat or heavily built the organ is often not clearly visible even if urography is performed. Then renal biopsy should be done in connection with renal angiography.

In cases of moderate uraemia (creatinine less than 6 mg per 100 ml of serum) repeated injections of contrast medium intravenously and injections of vasopressin subcutaneously will sometimes suffice to render the kidneys visible on the television screen. If necessary, arterial angiography is performed to display the kidneys in these patients.

Patients suffering from chronic renal failure and advanced uraemia often have arterial hypertension and a considerable bleeding tendency. At arterial renal angiography the vessels as well as the renal parenchyma are displayed. This augments the possibility of performing the biopsy in an area free from large arteries, although the kidneys often are shrunken and the renal cortex considerably reduced in thickness.

Two of us (Lindqvist and Nystrom) have used retrograde pyelography as a localizing aid when performing percutaneous renal biopsy in seven patients with advanced uraemia whose kidneys could not be seen directly at fluoroscopy (7). Because the contrast medium remains in the renal pelvis for a considerable time the operator is given ample time to watch the biopsy needle move together with the renal pelvis as the patient respirates. There is also time to place the tip of the needle in a position immediately beneath the renal capsule ensuring the best chance of obtaining an adequate specimen. Anyhow, as the method always carries a risk of introducing bacteria into the renal pelvis and any acute infection of the kidney in a patient with advanced uraemia will have serious consequences, we have now abandoned retrograde pyelography as a means of localizing the kidney at renal biopsy and prefer to use selective arterial angiography instead. However, we think that retrograde pyelography can still be used to aid in a biopsy attempt if a catheter must be passed into the renal pelvis for diagnostic reasons e.g. to exclude any postrenal obstacle in a patient with acute renal failure and anuria and where selective renal angiography is

not planned. Also when lacking facilities at renal angiography or when the procedure is contraindicated (hypersensitivity to the contrast medium) retrograde pyelography merits consideration as a method to visualize the kidney when renal biopsy is to be carried out

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THE OBSERVER VARIATION IN THE MEASUREMENT OF ARTERIAL BLOOD PRESSURE

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Abstract Observer errors and observer variation have been studied in a survey of blood pressure covering about 70 000 subjects in Bergen Norway carried out by 19 specially trained nurses under conditions standardized as much as possible. Substantial differences were found between extreme observers concerning the mean values of the readings in age and sex groups while the majority only showed insignificant differences. Reading characteristics were supposed to be the reason for individual observers keeping the same ranking position vis à vis the others from age group to age group in both sexes. A number of observers had however a different ranking in systolic from that in diastolic readings. Terminal digit preference in the readings was found only to a small degree and only in a few of the observers. The special apparatus of the London School of Hygiene was found to reduce but not to eliminate this error. When the results obtained with the latter apparatus were compared to standard apparatus results no differences in the reading characteristics were found related to the sex, age and blood pressure level of the subjects examined.

Conclusion observer variation in blood pressure examination may be substantial and may affect the mean values found in population surveys even if the observers are carefully selected and thoroughly trained.

The measurement of arterial blood pressure is a valuable method of assessing the condition of the heart and circulatory system in individuals and groups. Because of its ease of application it has been used extensively in epidemiological studies which have contributed greatly to the knowledge of the cardio-vascular conditions in population groups all over the world.

The evaluation of the blood pressure findings has however been complicated by substantial experimental error (5). Clearly this error has to be kept in mind in the practical use of blood pressure readings and in the planning of epidemiological studies.

The Bergen Blood Pressure Committee is constituted of members from the University of Bergen, School of Medicine, Medical Departments A and B and Bergen Helseråd.

Present knowledge indicates (6-8) that the true blood pressure undergoes variation depending upon factors such as sleep, physical activity, emotional status and environmental influences. In addition other factors influence the indirect measurement of the pressure: details of the instrument used such as the length and width of the cuff, the rate of inflation and deflation of the cuff, the position of the arm measured are important (5, 7, 9). Technical details of the sphygmomanometer such as the reading column may result in some readings being too high and others too low and in terminal digit preference (7). The technical competence of the person performing the examination will also influence the results. Mental concentration, reaction time, hearing acuity, interpretation of sounds, visual details and the separation of auditory and visual cues in the observation of a moving mercury column may all be assumed to be of importance (3, 7). The relationship between observer and subject may be relevant and different results according to age, sex and other characteristics of the observer may be obtained. Such factors may substantially influence the blood pressure readings in individuals and may also distort the frequency distribution of group values and affect the mean values (7). Consequently the errors made should be known and if possible avoided or corrected for. This will be of special importance in population studies where the blood pressure is measured by several observers. Here the variation between observers should be recognized.

MATERIAL AND METHODS

A blood pressure survey was carried out in the population of Bergen, Norway 1963/64. The objects of the

survey were several and included a study of the inter personal variation in the measurement of arterial B.P. At the same time precautions were taken to reduce observer variation as much as possible.

Outline of the survey

Measurements of B.P. weight and height were carried out in connection with mass radiography of the chest at several survey centres mostly schools in the city of Bergen. The B.P. was measured last and the subjects were allocated at random among the observers. Care was taken to rest the subject in a standardized position. The initial pulse rate was noted. In the subsequent auscultatory recording of the B.P. the following readings were taken:

- 1 Systolic pressure at the first appearance of the Korotkoff sound
- 2 Diastolic pressure 4th phase at the point of the muffling and
- 3 Diastolic pressure 5th phase at the point of disappearance of the sound

In a 10 per cent random sample of the entire population of Bergen 14 years and over chosen in advance of the study the special apparatus constructed by Rose, Holland and Crowley model Mark 3 (7) was used in addition to the conventional sphygmomanometer for each subject. The two instruments were used in a strictly alternating order from subject to subject and the same cuff was used for both instruments 14 by 40 cm.

In all 70 445 subjects (42 550 females 27 895 males) 15-100 years of age had their B.P. measured. The random sample consisted of 8794 subjects examined (4658 females 3636 males).

Observers

The B.P. was measured by registered nurses. Among those who volunteered for the project, 19 were accepted. These 19 nurses had the following age grouping: 20-29 years of age two, 30-39 seven, 40-49 five, 50-59 four and 60-69 one.

The nurses were thoroughly instructed during a training period of two weeks. Basic information about the testing procedures was given and practical examinations were carried out under close supervision both on healthy subjects and on hospital patients. In one nurse an audiogram was taken to test her hearing. In addition, all the nurses were tested with a tape recording (7) of the sounds of 12 subjects with varying B.P. characteristics and with a pre-fixed "true" reading of each compared to a scale in seconds measured with a stop-watch. Thus a record of individual reading abilities was obtained which was later used for comparison both half way through the survey period and at the end of the survey.

The study was planned so as to classify the results from each nurse according to the time of day, stage of study period, place of examination and age and sex of the subjects. When both types of sphygmomanometer were used, these same details were noted and in addition the results from the two instruments were compared.

Detailed printed instructions were given to each nurse. In the first part of the study period the nurses were supervised to ensure that the instructions were adhered to and that no misunderstanding prevailed.

Table I The number of subjects examined by each observer

Age groups 15-100 years

Observer	♂	♀	Total
A	2 207	3 258	5 465
B	647	1 261	1 908
C	750	1 347	2 097
D	1 941	2 972	4 913
E	2 190	2 880	5 070
F	1 359	3 187	4 546
G	2 083	3 162	5 245
H	1 103	1 644	2 747
I	2 050	3 264	5 314
J	839	1 284	2 123
K	1 316	1 918	3 234
L	2 066	2 936	5 002
M	668	892	1 560
N	1 469	1 586	2 855
O	2 376	3 486	5 862
P	753	1 333	2 086
R	973	1 385	2 358
S	1 131	1 567	2 698
T	2 174	3 188	5 362
Total	27 895	42 550	70 445

RESULTS

Blood pressure values among observers

The total results of the blood pressure readings for each observer were studied: the systolic and the two diastolic pressures separately. Considerable differences were found between the observers but much of this difference was due to the different age and sex composition of the groups examined by each nurse. Consequently it was considered necessary to compare sex and age specific values only.

Table I gives the number of subjects examined by each observer varying from 1560 to 5862.

Here as well as in the figures and in the appendices the observers are identified by letters from A to T. The position in the alphabet has been made on the basis of the total deviations by each observer from the group mean values of the systolic blood pressure of the different age groups.

In appendices I-IV the mean values of the 3 blood pressures, the standard deviations and the numbers examined are listed for each observer according to sex and age groups. Only the ages 30-69 years are presented due to shortage of space. (The results of the other age groups may be obtained from the authors.) In these tables it may be seen that for systolic blood pressure some

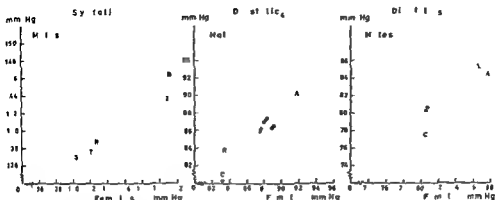


Fig 1 Correlation of mean blood pressure readings by each of 19 nurses in males and females age 50-59 years

of the observers have mean readings differing from the group mean value by as much as 5-11 mm Hg. There is clearly a tendency for some observers to have readings higher than the group mean in all age and sex groups while others have low readings in all groups. The difference between extreme observers is considerable. Thus in the age group 60-69 years in females observer B has a mean reading of 171.5 mm Hg while observer S has 152.9. In the youngest group 20-29 years observer H has the highest reading in males of 132.5 mm Hg while observer S has 123.7.

In the two diastolic pressures some of the observers deviate from the group mean by as much as 8-9 mm Hg but most of the deviations are between 0 and 4 mm Hg. The greatest difference between extreme observers amounts to 95.2-81.7

mm Hg for observers P and C in diastolic 4th phase and to 88.7-74.8 mm Hg for observers A and C in diastolic 5th phase in the age group 70-79 years. In the youngest age group in males the greatest difference in the 4th phase amounts to 81.0-67.5 mm Hg in observers H and O and in the 5th phase to 74.1-64.0 in observers L and J.

It may be seen that in systolic readings the observers tend to keep the same relative order within the group of observers from age group to age group and between the sexes.

In the diastolic readings the tendency to relative positioning within the group is different from the systolic and is not consistent to the same degree. However as an example it may be mentioned that observer A read higher than the group mean

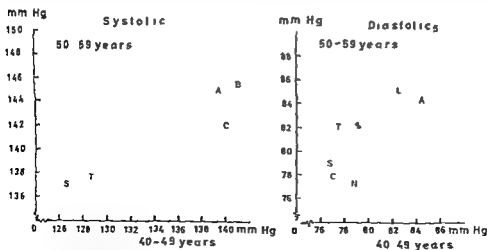


Fig 2 Correlation of mean blood pressure readings by each of 19 nurses in males age 40-49 and 50-59 years

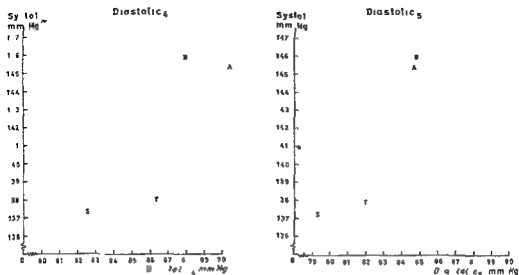


Fig 3 Correlation of mean systolic and diastolic readings by each of 19 nurses in males age 50-59 years

in all age groups for systolic as well as for the two diastolic pressures while observer S read lower in all three pressures. Observer C read systolic pressure higher than the group mean while she read the two diastolic pressures lower than the group mean. Observers F, G, I, and M maintained an intermediate position in all readings.

In Fig 1 the age group 50-59 years has been chosen to demonstrate the correlation between the readings in males and females for each observer. The linear correlation coefficient between

the mean values for each observer is 0.76 in systolic, 0.89 in diastolic 4th phase and 0.85 in diastolic 5th phase pressure.

Fig 2 shows that the reading of each observer in one age group correlates quite well with the readings in another age group of the same sex. The linear correlation coefficient in males is 0.71 in systolic, 0.70 in diastolic 5th phase pressure while in females the coefficient is higher, 0.83 and 0.95.

Fig 3 demonstrates that the readings of systolic and diastolic values are reasonably well correlated.

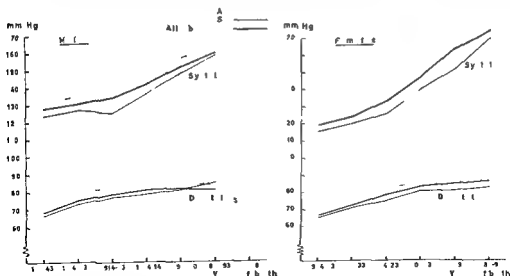


Fig 4 Systolic and diastolic blood pressure in age groups. Mean values for observers A and S and for all observers.

in each observer. The correlation coefficient of systolic blood pressure to diastolic phase 4 is 0.68 and to diastolic phase 5 0.72. In females of the same age group the coefficients are 0.65 and 0.72 respectively.

The difference between extreme observers A and S is illustrated in Fig. 4 where the mean values of systolic and diastolic 5th phase pressure are compared with those of the total of the 19 observers.

The variation of blood pressure readings in each observer

The variation of the readings in each observer is indicated by the size of the standard deviation in each age and sex group for the 3 blood pressures which may be seen in appendices I-IV. No definite trend emerges to indicate a real reading differences between the observers.

Even if differences between the observers regarding variation of the readings may be partly caused by actual reading differences they may also be influenced by the variations in the actual pressures between the subjects examined. Consequently in this material a closer study of the standard deviation does not form sufficient basis for differentiation between observers as regards reading stability.

The influence of the sex of the subject examined

In appendices I-IV it may be seen that in each observer the magnitude of the difference from the group mean value is about the same for males as for females. Fig. 1 also indicates no great difference between the readings in the two sexes. There is therefore no tendency to obtain different readings in males from those in females and there is no indication of influence of age, charm or personality of the female observers. This point will be studied below using a different approach.

Terminal digit preference

Preference for certain terminal digits in the readings by the different observers has been investigated. In the total group of observers a small preference is indicated for zero values in the use of the conventional apparatus to measure systolic blood pressure.

Digits	0	1	2	3	4	5	6	7	8	9
Percentage	25	18	20	17	20					

When the special apparatus of Rose, Holland and Crowley is used the digit preference is much less.

Digits	0	1	2	3	4	5	6	7	8	9
Percentage	19	8	11	8	10	9	11	8	12	8

In this apparatus however the preference for the zero value is also indicated and the uneven digits are slightly underrepresented.

In the individual observers appendices V and VI demonstrate a much greater variation in digit preference. Observers L and S have a definite preference for zero in the use of the conventional apparatus. Observers B, H, J and O show the same preference to a lesser extent. In the others no definite digit preference is found. Oddly enough some of the observers also show some digit preference in using the special apparatus. This is particularly so for observers D and S and to a lesser degree for observers K, M and R.

The tendency demonstrated here for the systolic blood pressure is also present for the diastolic 4th phase. In appendix VI it may be seen that observers D and S have preferred the even digits in the use of the special apparatus.

Differences between results according to type of apparatus used

In the 10 per cent random sample of the adult Bergen population the two types of blood pressure apparatus used for each subject allow for several comparisons of the reading characteristics of each observer. The special apparatus is supposed to give readings with less reader bias such as terminal digit preference and overestimation of certain values. Thus the results of the special apparatus may be used as an indication of the true blood pressure at the particular moment and under the particular circumstances. Consequently the difference between the results obtained with the two apparatuses may give an indication of the accuracy of the reading when the conventional apparatus has been used.

However the chosen procedure of alternating using the special (S) or the conventional, ordinary (O) apparatus first influences the results. The time necessary for the first reading represents a period of rest, which in turn may give a lower reading with the second apparatus. This period of rest may be longer when the more com-

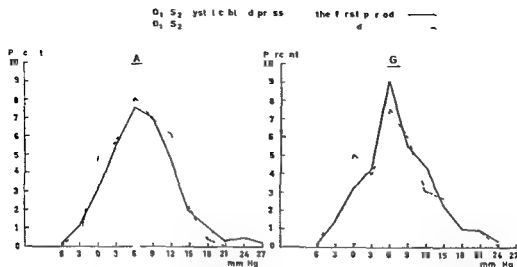


Fig 5 Comparison of differences between systolic blood pressure measured in the first and the second half of

the work period each day with the two apparatuses in the same individuals—observers A and G

plicated Π apparatus is used first. In addition to such a rest factor an apparatus factor may be present because the S apparatus tends to give lower systolic readings than the O apparatus.

The difference between the results when either the S or the O apparatus is used first may be listed:

O-S apparatus factor plus rest factor (which is small short time)

O₂-S apparatus factor minus rest factor (which may be longer than the above due to longer rest period)

Consequently the differences between the results of the two apparatuses will be greatest when the ordinary conventional apparatus is used first and the special apparatus second. As the rest factor is supposedly of greater importance in systolic than in diastolic blood pressure the difference between the two differences listed above would be expected to be greatest in systolic values.

Several of the possible reader characteristics have been studied by using the differences between the results of the two apparatuses just mentioned.

Influence of sex

In the majority of observers the influence of the sex of the individual examined is found to be insignificant using this method of investigation. In observer N, the males have greater apparatus differences than the females—approximately 5 mm Hg for the systolic, 3 mm Hg for the diastolic values. In observer F the females have greater differences than the males but only approximately 2 mm Hg.

Thus also using this method of study the majority of observers do not differ much from

each other whether they measure males or females. The sex component seems altogether to be a very small part of the experimental error.

Influence of age

The importance of the age of the subject examined has been analysed in the same way among the observers. There is no difference in this respect between the results of the two apparatuses used. Consequently it may be assumed that no systematic error depending upon the age of the subjects examined exists among the observers.

Influence of fatigue

The same method has been used to study the effect of fatigue on the reading of blood pressure by individual observers during their working period which lasted 3–6 hours. A rest of 15 minutes was taken whenever this was felt necessary by the observer and when the conditions permitted. The difference between the mean results of the two apparatuses in the first and second half of the daily working period has been used for this investigation. Practically no difference is found between the results from the two periods. Fig 5 gives an illustration of this comparing the frequency distributions of the differences in two particular observers A and G.

Thus the reading characteristics do not seem to have been influenced much by fatigue in the observers (Each observer examined between 1560

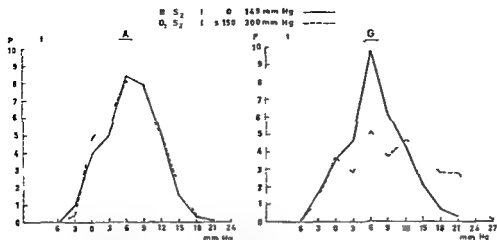


Fig 6 Comparison of differences between systolic blood pressure measured with the two apparatuses in the same

individuals grouped in values above and below 150 mm Hg—observers A and G

and 5862 subjects during the study period of approximately 4 $\frac{1}{2}$ months giving a number of 20–50 examinations per day. In one hour the number of examinations per observer was 1 to 15 (with the greatest load occurring during particular “rush” hours).

Influence of the height of the blood pressure

The differences between observers in reading characteristics may depend upon the height of the blood pressure measured. Differences between the results of the two apparatuses were therefore studied with a systolic blood pressure grouping of less than 150 mm Hg compared to 150 mm Hg and above. In this analysis no great influence of the height of the blood pressure in this respect was demonstrated either in the individual observer or between observers. As an illustration of the comparisons carried out, Fig 6 gives the frequency distributions of the differences between the values obtained with the two apparatuses in the same subjects comparing observers A and G. It may be seen that observer A demonstrates almost identical curves for the two blood pressure levels. The same was found for the majority of the other observers as well. Observer G clearly shows different distributions in the two pressure levels. This was also found when she used the special apparatus first.

The reason why observer G had relatively large differences between the results of the two ap-

paratuses in her high pressure group may be that she used a long time for her examination giving some of the “artificially” high pressures time to decrease in the second reading. Chance factors may also have been involved and she may have had particularly large numbers of subjects with high but labile systolic blood pressure.

Altogether the differences and similarities between the 19 observers illustrated above may partly depend upon individual reading characteristics but may also of course be a result of true differences between the subgroups examined by each observer. The latter factor may be reduced by the large number of subjects examined and the use of the mean values specific for sex and an age grouping of ten years. But this factor of “true” differences between the subjects measured cannot be disregarded.

The ideal method for comparing the observers would be to let them examine the same subjects under standardized conditions. This ideal was aimed at in the application of the tape recording test the results of which are reported in (4).

DISCUSSION

The Bergen Blood Pressure Survey of 1963/64 has covered a group of nearly 70 000 subjects in 4 $\frac{1}{2}$ months. Such a number of blood pressure readings in so short a period requires a large team of observers. Variations in reading tech-

nique and in individual factors may produce differences between the observers. It may be of interest to study these differences and their influences upon the total results.

First of all the selection of observers for the blood pressure examinations is important. In the Bergen survey qualified nurses were chosen. The reason for this was partly the success of using nurses in the survey of 1950/51 (1) and partly the impossibility of getting sufficient numbers of physicians to carry out the work. Several surveys have utilized nurses or technicians for the measurement of blood pressure (1, 2) and their performance has been considered as good as if not better than that of physicians (3, 7).

In the present study two of the nurses who volunteered for the examinations were more than 55 years of age. In one of these it was found necessary to have an audiogram taken to ensure that her acoustic sense was adequate. Otherwise the two older nurses were efficient enough during the testing and training period to warrant their inclusion in the team. During the survey the older nurses had somewhat greater difficulties than the younger ones during rush hours with many examinations. That they showed no influence of fatigue however may be explained by the routine rest period and perhaps particular care shown by their colleagues.

The analysis of the results of these two nurses, observers L and N, showed that observer L tended to have very high readings of diastolic 5th phase pressure while she had intermediate systolic and diastolic 4th phase readings. This may be associated with her slightly deficient hearing. Observer N tended to have very low diastolic values which may be explained by good hearing or long reaction time. The latter explanation is supported by her results in the tape recording test.

However the two older nurses are not significantly different from the others in their total accomplishment. Among the other nurses too facility for blood pressure reading could not be graded on the basis of age in the findings of the present survey. Otherwise none of the nurses had had any great experience in blood pressure measurement before the survey. Consequently their abilities had to be judged during their period of training; the training had to be very thorough and detailed as were the written instructions.

Even when sex and age specific values were

used the total results demonstrated some differences between the observers. In systolic pressure the greatest difference was to be found between observers U and S in females aged 60-69 years. This difference amounted to 18.6 mm Hg. In diastolic pressure the greatest difference was found between observers P and C in females aged 70-79 years where it was 13.5 mm Hg. In diastolic pressure the greatest difference was between observers A and C in females aged 70-79 years and was 13.9 mm Hg. These differences between these particular observers were 5-6 times the standard error of the difference between means. Apart from the extreme observers the differences between observers and between individual observers and the group mean were less than twice the standard error.

Of course the composition of each sex and age specific group examined by each observer may influence the mean values and explain the inter-observer differences. But when these differences are of the same size and direction from age group to age group in both sexes, one may assume that there is an actual reading variation between observers. Consequently there is justification in studying the individual observer characteristics concerning blood pressure reading by comparing sex and age specific values. Thus a ranking list may be made out in which the position of any particular observer may be studied for the 3 blood pressures.

Observer A is top of this list reading all 3 pressures higher than her colleagues. Observer S is at the bottom when all 3 pressures are taken as a whole—even if the positions at the bottom change somewhat from one pressure to the other. The rest of the observers occupy partly the same intermediate ranking positions but some have also been found to vary considerably between the 3 pressures. This reading variation between the observers influences the mean values of the total results but with the great numbers examined in the present study there is no distortion of the distribution curves.

Other reading characteristics are demonstrated in this survey. The terminal digit preference is small when the total results for all observers are investigated. But when the totals are split according to each observer definite differences appear. The preference of the zero value is clearly present in this study as in so many others but

the zero does not dominate to the same extent as in other reports (1 2 5 7)

In those observers where terminal digit preference is pronounced it is consistent in all 3 blood pressures. It might be expected that the systolic reading with its quite definite hearing point would be less liable to this bias than the 2 diastolic readings but this is not so in the present study.

The special sphygmomanometer of Rose Holland and Crowley clearly reduces the reading errors represented by the terminal digit preference but in three observers in the present study D P and S preference for even digits is obvious. Observer S also has a pronounced zero-preference in the use of the conventional apparatus while the preference of observer D is mainly present in the use of the special apparatus. But here it is consistent in all 3 pressures.

Owing to the construction of the special apparatus it is very surprising that these results of observers D P and S should occur. In observer D only 16% of the readings have an uneven terminal digit in observer S 9%. The number of readings should be large enough to reduce the risk of pure chance results of that kind. The explanation may be that a "writing bias" has been introduced. But a closer study shows that this bias must have been present when the special apparatus was used first as well as second and at all times of the day and at all blood pressure levels. It may be that the bias arises because of the combination with the measurements of the conventional apparatus where only even digits are present. In any case it must be assumed that the observers D P and S have not followed the detailed printed instructions for the reading and subsequent writing of the blood pressure measured with the special apparatus.

The part played by fatigue in the experimental error in blood pressure measurement under population survey conditions does not seem to have been great in the present material. Some explanation of this point may be found in the design of the survey. The number of observers in relation to the number of subjects to be examined the routine of alternating work and rest the number of hours at work per day and all the other details of the work were planned on the basis of careful calculations and considerations—in order to avoid too much stress upon the observer nurses (and simultaneously upon the subjects to be

examined). That no significant differences may be found between the reading results from the two halves of the daily working period may be taken as an indication that these efforts at fatigue prevention were successful.

The influence of the age and sex of the subjects examined and the level of the blood pressure measured have not had much effect on the reading error. These factors have partly been studied with the aid of the differences between the readings with the two sphygmomanometers used but this study basis may be ill suited for the purpose. However the care taken in the blood pressure readings the relatively great number of readings and the consistent findings in this respect for all 3 blood pressures give support to the conclusions.

The use of the two sphygmomanometers in the 10 per cent random sample of the adult Bergen population as a part of the present study has been of importance in several ways. The apparatus factor thus introduced has made possible a correction of the readings with the conventional apparatus. At the same time the observer nurses became interested in the readings and were anxious to avoid faults. These conditions may however have introduced a factor of bias in the reading.

The terminal digit preference has been definitely reduced in the use of the special apparatus as previously mentioned. Pressing of the three buttons has not presented any problems and the inspection of the mercury columns and levelling of the cursors to the meniscus have had the advantage of giving sufficient time for accurate readings. The constant rate of deflation of the cuff pressure was an important part of the standardized procedure (7).

The use of an additional apparatus has required additional time and usually rest for the subject to be examined. Consequently this procedure itself may give lower blood pressure values especially for the systolic pressure. Thus the blood pressure findings will be somewhat lowered in the group in which both instruments were used. This will affect the mean values and will place the frequency distribution slightly more on the side of the lower values than if only one apparatus had been used. This point however has no influence upon the problem of the particular study here presented.

The results of the differences between the mean

values of systolic and diastolic blood pressures of the individual observers in this series agree quite well with the findings of Lowe and McKeown (5). These authors found consistent and substantial differences between readings by 12 physicians of approximately 12 mm Hg between the highest and the lowest readings.

When comparing the mean deviations of each observer in this series with those found by Comstock (2), the mean difference in systolic pressure is found to be of almost the same magnitude except for observers R and T and partly A and M. Further comparisons are not being made because most other studies do not give a relevant basis for such comparison.

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Appendix I Numbers examined (n) mean blood pressure (x) and standard deviation (s) in males and females born 1894-1903 per observer

Ob- server	Males							Females						
	Systolic			Diastolic ₁		Diastolic ₂		Systolic			Diastolic ₁		Diastolic ₂	
	n	x	s	\bar{x}	s	\bar{x}	s	n	\bar{x}	s	\bar{x}	s	\bar{x}	s
A	297	158.5	28.3	90.1	15.0	84.2	15.0	50	169.9	30.9	91.4	13.7	88.8	14.2
B	81	156.8	28.5	87.8	13.5	84.5	13.6	155	171.5	29.0	91.2	14.1	86.8	15.7
C	9	162.2	27.3	84.7	14.2	80.7	15.6	170	166.4	27.4	82.7	14.5	79.6	14.6
D	57	153.4	27.0	86.5	13.6	81.9	14.2	409	167.3	31.0	92.6	14.3	86	14.5
E	301	154.0	25.8	86.1	12.4	80.3	12.5	397	164.5	27.9	89.6	13.7	83.1	13.4
F	291	154.1	27.6	86.7	13.5	82.4	13.8	509	169.5	29.9	92.9	14.2	88.3	14.5
G	30	154.5	26.3	87.2	14.0	81.3	14.6	438	167.2	29.8	90.5	14.5	83.4	14.7
H	218	156.9	27.6	89.9	12.9	84.8	13.2	380	164.1	28.5	91.3	11.6	85.8	12.4
I	291	153.6	27.5	86.8	14.5	81.8	14.7	501	165.1	29.8	90.0	13.2	84.6	13.4
J	11	153.0	27.0	87.4	12.7	81.1	12.4	159	165.4	30.7	90.7	14.4	83.7	15.5
K	260	154.3	27.7	84.8	12.0	80.4	12.7	497	166.8	30.7	88.3	13.0	83.3	13.5
L	313	152.4	24.0	87.3	13.2	85.4	13.6	478	161.9	26.7	90.4	13.2	88.4	13.7
M	97	154.9	31.1	86.5	14.4	83.7	13.9	105	158.3	26.8	86.1	13.1	81.7	15.7
N	272	154.0	27.5	86.1	13.5	77.5	14.7	359	163.0	31.4	86.4	13.3	79.5	13.3
O	338	155.7	27.5	86.0	13.2	82.1	13.6	517	165.3	31.8	89.1	13.8	85.1	13.8
P	117	146.8	23.1	87.5	12.3	83.0	12.5	210	164.6	26.4	93.7	13.9	87.6	15.0
R	119	146.3	22.5	83.2	10.6	80.5	11.0	144	165.1	31.3	89.3	14.9	85.0	14.7
S	116	148.7	25.9	85.8	13.0	82.5	13.5	188	152.9	27.9	84.7	13.4	81.2	13.4
T	303	144.0	23.1	86.4	12.8	82.3	13.4	465	156.9	29.2	91.5	14.0	86.3	15.6
Total	4146	153.5		86.7		80.2		6383	164.8		90.2		85.2	

Appendix II Numbers examined (n) mean blood pressure (x) and standard deviation (s) in males and females born 1904-1913 per observer

Ob- server	Males							Females						
	Systolic			Diastolic ₁		Diastolic ₂		Systolic			Diastolic ₁		Diastolic ₂	
	n	x	s	\bar{x}	s	\bar{x}	s	n	\bar{x}	s	\bar{x}	s	\bar{x}	s
A	381	145.5	23.9	90.1	15.0	84.4	12.7	566	150.0	6.3	91.3	13.0	87.2	12.9
B	18	146.0	8.5	87.6	10.8	84.4	11.4	211	151.8	24.8	87.9	11.7	84.0	12.0
C	138	146.3	21.8	80.5	11.4	78.0	11.3	23	149.3	21.3	83.4	11.1	80.3	11.8
D	336	145.2	24.4	86.5	13.6	83.4	13.0	492	149.2	25.5	89.1	12.2	83.8	13.3
E	376	143.8	21.8	86.1	12.4	80.4	11.8	495	149.4	25.7	87.7	12.9	81.8	13.1
F	246	145.4	22.4	86.4	11.7	82.0	12.6	434	148.7	7.2	89.0	12.0	85.0	12.1
G	382	145.6	24.0	87.2	13.5	81	13.5	581	148.8	5.5	88.2	12.7	83.0	13.2
H	190	143.8	23.3	87.3	11.6	82.9	11.4	308	148.9	26.4	88.3	10.8	84.4	11.3
I	415	143.8	23.3	85.6	10.8	81.1	11.1	539	150.5	6.6	88.0	12.4	83.4	12.7
J	135	145.2	22.8	85.9	10.8	79.3	12.1	219	145.9	25.2	87.1	12.7	81.8	13.1
K	29	143.1	22.6	84.8	11.0	80.6	11.8	35	146.8	5.8	84.8	11.3	80.9	11.7
L	356	144.6	21.2	87.0	11.9	85.1	13.6	503	146.4	3.7	88.1	13.0	86.2	13.3
M	118	145.0	22.3	85.7	11.3	84.1	11.3	151	145.1	22.0	84.9	11.5	83.1	12.1
N	235	141.0	26.6	82.8	11.7	77.6	11.6	349	147.2	5.5	84.7	12	79.1	11.9
O	421	139.0	21.5	82.6	11.2	79.7	11.9	685	146.6	25.1	86.0	12.0	87.8	12.2
P	143	143.0	24.9	87.6	13.3	82.2	13.7	233	145.2	4.6	89.7	13.1	84.9	13.3
R	173	138.6	23.8	83.5	11.9	81.1	11.6	23	141.1	23.7	84.1	11.1	81.1	11.4
S	189	137.5	20.2	82.7	10.1	79.0	10.4	277	140.4	23.4	84.1	12.6	80.7	12.5
T	364	138	22.6	86.0	12.6	81.7	12.5	541	141.9	6.3	87.6	12.7	83.4	12.9
Total	4955	141.9		85.7		81.7		7382	147.5		87.4		83.3	

Appendix III Numbers examined (*n*) mean blood pressure (*x*) and standard deviation (*s*) in males and females born 1914-1923 per observer

Ob server	Males							Females						
	<i>n</i>	Systolic		Diastolic ₄		Diastolic ₅		<i>n</i>	Systolic		Diastolic ₄		Diastolic ₅	
		<i>x</i>	<i>s</i>	<i>x</i>	<i>s</i>	<i>x</i>	<i>s</i>		<i>x</i>	<i>s</i>	<i>x</i>	<i>s</i>	<i>x</i>	<i>s</i>
A	449	139.3	19.0	88.3	11.7	83.8	12.2	571	137.3	21.2	85.5	11.6	81.7	12.2
B	113	140.7	18.2	85.6	10.9	81.3	11.5	190	138.3	21.0	82.9	11.4	79.4	11.8
C	130	139.6	17.3	80.5	11.1	77.6	11.6	195	134.4	18.6	77.5	10.4	75.2	10.9
D	393	134.9	17.3	85.4	10.3	80.8	10.8	565	134.8	20.6	84.0	11.2	79.0	11.6
E	476	135.7	17.7	83.3	10.5	77.6	11.0	554	136.3	21.3	82.9	11.4	77.5	11.9
F	191	135.7	20.0	85.6	12.1	81.1	12.4	323	133.7	17.8	84.5	10.5	80.4	10.9
G	380	136.6	18.5	84.6	11.3	79.2	11.8	570	135.9	20.6	82.9	11.0	78.2	12.0
H	151	137.2	20.9	86.8	10.8	81.7	11.3	189	133.1	18.2	83.7	10.1	79.4	10.5
I	420	136.7	18.6	84.2	10.1	79.5	10.4	579	133.8	18.7	82.7	10.1	78.4	10.3
J	145	134.9	17.1	85.1	10.5	78.5	12.3	186	134.8	19.2	83.7	9.9	77.1	11.5
K	206	131.8	16.0	80.4	9.1	76.2	9.6	277	135.8	21.0	81.5	11.1	77.2	11.2
L	381	136.4	16.4	84.0	10.4	82.0	10.6	528	133.4	18.3	83.0	11.2	81.0	11.4
M	153	135.1	18.4	81.7	11.1	77.8	13.8	160	135.3	21.7	81.2	12.3	78.3	13.1
N	163	136.9	19.6	82.9	11.2	78.1	11.2	207	132.7	21.0	80.2	11.3	75.6	11.3
O	410	131.9	19.2	80.8	10.7	77.3	11.4	602	132.1	20.6	80.2	10.9	76.9	11.1
P	140	131.6	15.6	83.9	9.1	79.1	10.8	242	137.1	19.1	84.7	11.4	80.3	12.4
R	205	132.5	17.5	80.7	10.3	77.2	10.5	279	131.5	18.0	80.2	10.2	77.7	10.4
S	169	129.6	16.4	80.3	10.1	77.4	10.4	221	126.4	18.2	78.6	11.3	75.0	11.6
T	411	178.6	19.0	82.7	10.6	77.8	10.8	567	128.7	20.5	82.6	11.5	78.1	12.3
Total	5056	134.9		83.7		79.3		7005	133.8		82.6		78.5	

Appendix IV Numbers examined (*n*) mean blood pressure (*x*) and standard deviation (*s*) in males and females born 1924-1933 per observer

Ob server	Males							Females						
	<i>n</i>	Systolic		Diastolic ₄		Diastolic ₅		<i>n</i>	Systolic		Diastolic ₄		Diastolic ₅	
		<i>x</i>	<i>s</i>	<i>x</i>	<i>s</i>	<i>x</i>	<i>s</i>		<i>x</i>	<i>s</i>	<i>x</i>	<i>s</i>	<i>x</i>	<i>s</i>
A	336	135.6	15.8	84.7	10.6	79.3	11.1	452	129.3	15.4	81.2	10.3	76.9	11.1
B	92	134.0	13.3	82.3	10.1	77.4	11.9	140	126.1	16.4	76.9	9.4	72.9	10.0
C	116	132.8	13.7	73.3	11.5	69.8	12.0	156	125.2	14.3	71.8	9.8	69.2	10.3
D	311	132.4	16.7	82.5	11.2	77.2	17.4	496	125.3	16.6	78.4	10.2	77.9	10.9
E	362	131.5	15	79.9	9.9	73.1	10.6	437	125.2	14.2	76.0	9.3	70.4	10.0
F	133	129.0	15.9	79.7	10.0	75.2	10.4	197	126.0	15.8	79.3	10.1	74.9	10.1
G	320	131.0	14.3	80.0	9.6	73.4	11.1	489	125.1	15.1	76.7	9.8	71.3	10.9
H	127	130.6	14.3	83.2	8.4	77.2	9.5	180	125.8	14.3	79.8	8.7	74.1	9.4
I	292	132.3	14.2	80.0	8.4	74.9	10.4	464	126.0	14.6	77.9	10.1	73.2	10.6
J	147	129.9	14.8	79.8	8.7	71.6	10.7	144	124.9	15.7	77.4	9.6	70.9	11.1
K	150	129.6	15.4	77.3	9.5	72.5	10.2	158	126.3	14.1	75.3	8.8	71.0	10.7
L	292	134.1	14.7	80.9	11.3	78.8	11.6	431	126.0	12.7	77.6	9.3	75.4	9.4
M	95	129.1	13.6	78.1	9.8	75.1	10.8	133	123.9	14.2	73.5	9.3	70.5	10.5
N	136	130.5	13.7	78.6	9.6	72.6	10.6	138	121.7	13.0	74.8	8.7	69.6	10.7
O	360	128.1	14.8	78.0	9.2	72.2	9.8	522	122.3	14.4	75.1	9.2	71.4	10.1
P	121	130.7	14.1	82.0	9.4	76.4	10.6	215	124.8	14.6	79.3	10.5	75.1	11.0
R	168	128.8	13.2	77.8	10.0	74.8	10.1	238	122.4	13.7	74.7	9.4	71.1	9.6
S	183	127.5	15.0	76.6	11.9	73.5	11.9	191	120.5	13.7	74.5	10.5	71.5	10.6
T	254	127.3	16.6	80.7	10.1	75.9	11.5	469	118.2	15.1	76.3	10.1	71.2	12.0
Total	4095	130.9		80.1		75.2		5650	124.5		77.0		72.6	

Appendix V *Distribution of terminal digits in the readings of each of 19 observers with the conventional and the special apparatus*

Systolic blood pressure

Ob server	Conventional apparatus ()					Special apparatus ()									
	0	2	4	6	8	0	1	2	3	4	5	6	7	8	9
A	25	13	20	23	19	14	9	11	11	10	8	8	7	12	10
B	28	16	19	21	16	14	6	6	5	9	10	15	14	14	7
C	17	26	19	17	21	9	6	12	8	13	10	9	9	14	10
D	15	11	27	16	24	15	2	18	3	17	2	17	2	27	2
E	26	24	22	13	15	9	9	8	8	10	12	11	11	12	10
F	15	25	18	22	20	13	9	11	10	9	9	10	8	11	10
G	15	20	21	24	0	11	12	9	8	12	8	11	10	10	8
H	31	15	20	14	20	21	7	14	5	10	10	10	7	10	6
I	19	20	20	19	22	12	11	9	9	6	12	10	10	10	11
J	31	15	17	15	22	10	7	12	8	12	12	11	8	11	9
K	11	24	20	0	23	3	20	8	14	4	13	4	14	8	12
L	42	16	17	12	13	18	7	8	8	9	9	9	10	11	11
M	24	14	22	17	23	23	6	10	9	8	3	13	8	15	5
N	28	17	23	18	14	27	5	18	6	11	10	10	4	11	4
O	29	19	21	12	19	11	7	12	9	12	10	10	10	11	8
P	23	19	21	19	18	15	6	17	7	12	8	13	9	16	7
S	56	13	6	4	21	27	2	11	2	10	3	17	5	18	5
T	18	18	17	22	25	16	6	13	7	12	9	10	8	12	8
Total	25	18	20	17	20	14	8	11	8	10	8	11	8	12	8

Appendix VI *Distribution of terminal digits in the readings of each of 19 observers with the conventional and the special apparatus*

Diastolic blood pressure

Ob server	Conventional apparatus ()					Special apparatus ()									
	0	2	4	6	8	0	1	2	3	4	5	6	7	8	9
A	28	16	20	23	13	10	9	10	10	9	10	12	9	12	11
B	33	16	20	15	16	9	13	11	8	12	9	8	11	9	9
C	10	16	20	20	14	11	8	9	8	14	10	9	10	14	7
D	21	16	1	14	25	16	2	18	2	17	3	17	4	20	5
E	38	17	17	13	15	9	6	10	9	10	9	12	11	10	14
F	28	17	17	15	23	7	8	9	11	10	11	10	9	11	14
G	20	16	23	23	18	12	9	9	11	10	9	8	12	10	10
H	46	8	18	14	14	13	6	11	10	10	9	10	12	12	7
I	22	14	22	20	22	10	12	9	8	7	9	10	11	13	11
J	37	16	24	12	11	10	11	10	10	11	12	8	9	10	9
K	30	10	20	15	15	10	12	3	13	13	8	11	10	8	12
L	29	16	20	14	11	14	6	13	10	10	12	7	10	8	10
M	22	21	27	18	12	16	6	18	7	9	7	14	6	11	10
N	48	16	22	7	7	18	7	9	10	12	9	9	7	13	6
O	33	16	20	9	22	12	9	14	6	9	9	10	11	9	11
P	39	13	22	16	10	18	2	15	9	16	7	12	3	15	3
S	50	12	19	7	12	23	1	12	1	15	2	21	2	0	3
T	15	26	19	14	26	13	7	10	8	18	13	10	9	10	8
Total	30	17	20	16	17	12	8	11	8	11	9	11	9	12	9

Table I Incorporation of DL mevalonate 2-¹⁴C and DL leucine 4-5-³H into serum free cholesterol in normal and hypercholesterolemic human subjects

Case no	Age (y)	Body weight (kg)	Serum triglycerides (mg/100 ml)	Serum cholesterol		Free ¹⁴ C-cholesterol		Free ³ H cholesterol	
				Total (mg/100 ml)	Free (mg/100 ml)	Injected dose at peak SA ^b (μg)	SA ratios (h/peak)	Injected dose at peak SA ^b (μg)	SA ratios (h/peak)
1	61	58.0	226	640	201	7.28	0.064	0.1350	0.039
2	35	64.0	244	519	170	2.61	0.109	0.03	0.338
3	31	72.0	100	310	104	3.41	—	0.0167	0.375
4	28	69.0	200	500	167	7.57	0.154	0.1170	0.443
5	31	80.5	500	504	141	6.10	0.049	0.0291	0.291
6	41	76.5	152	261	72	3.91	0.063	0.0332	0.446
7	43	66.0	75	234	57	2.55	0.137	0.0274	0.714
Mean			200	427	125	4.78	0.096	0.0541	0.347
± s.e.						± 0.82	± 0.01	± 0.0188	± 0.056

Plasma volume was assumed to be 4.5 of body weight

^b SA = specific activity (dpm/mg)

C (both used as produced by The Radiochemical Centre Amersham England) was prepared in dilute ethanol and a known amount (80 μC of H and 10 μC of C) was administered intravenously. Blood samples were then obtained 1 2 4 8 24 and 48 h later. Serum was separated and kept frozen before analysis. Extraction of lipids was carried out by 10 volumes of ethanol ether (1:2). Esterified cholesterol was separated from free cholesterol and triglycerides by thin layer chromatography on silica gel G by developing the chromatoplates with heptane:ethyl ether:acetic acid (80:20:1). After extraction of lipid classes, fatty acids of the cholesterol ester fraction and of the free cholesterol fraction (containing diglycerides) were removed by saponification for 1 h at 45°C in ethanolic (90%) NaOH (1M) solution. Water was added to bring ethanol concentration to 60% and cholesterol was extracted with petroleum ether. Cholesterol was measured according to Hansen and Dam (9) and triglycerides by the method of Carlson (7). Radioactivity was counted in a Packard Tri-Carb Liquid Scintillation Spectrometer using external standard for the determination of the absolute activity levels (dpm). Serum α-ketoglutarate level was measured according to DeSchepper et al (5).

The fractional turnover rate of esterified cholesterol was calculated both for ³H and ¹⁴C cholesterol by plotting the specific activities of the free and esterified cholesterol on cartesian graph paper. The calculations were carried out as presented by Zilversmit (14) assuming that serum esterified cholesterol is derived from free cholesterol in the plasma or liver. The validity of the method for the turnover rate of serum esterified cholesterol is discussed in detail by Nestel and Monger (19). They assumed that the small amount of esterified cholesterol present in the liver (less than 10% of the plasma pool) causes an insignificant error in the results.

RESULTS

Incorporation of ¹⁴C mevalonate and ³H leucine into serum free cholesterol

Table I shows that up to 8% of injected DL mevalonate 2-¹⁴C and only from 0.017 to 0.135% of DL leucine-4-5-³H were converted to serum free cholesterol at its peak specific activity. The plotting of these values against the pool size of free cholesterol according to Fig. 1 demonstrates particularly for ¹⁴C cholesterol that the larger the serum pool the more of the label is found in serum free cholesterol.

That the rate of the appearance of the two labels in serum free cholesterol is not the same is illustrated by the ratio ¹⁴C/³H in Fig. 2. The ratio increases rapidly reaching its maximum at 4 h and falls thereafter slowly. This suggests that shortly after administration of the labels

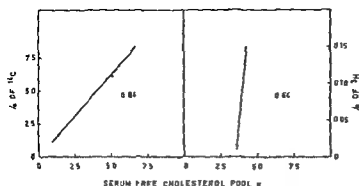


Fig 1 Correlation of the serum free cholesterol pool to the radioactivity (of dose) found in serum free cholesterol in the peak specific activity after simultaneous administration of DL mevalonate- ^{14}C (left side) or DL leucine-4,5 ^3H (right side) intravenously to human subjects

cholesterol synthesized from leucine entered into the circulation faster than that formed from mevalonate. A slow decrease of the ratio after 4 h (at 48 h all values were lower than at 4 h) indicates on the other hand that the release of ^3H -cholesterol into the circulation is more prolonged than that of ^{14}C -cholesterol. This may be due to recirculation of ^3H -compounds derived from ^3H leucine or release of ^3H -cholesterol from tissues which synthesized less ^{14}C -cholesterol. The faster rate of increase and the slower rate of fall of the specific activity of ^3H cholesterol as compared to ^{14}C cholesterol is seen also in the ratios 1/h and 4b/h to the peak specific activity (Table I). These ratios for ^3H -cholesterol were in general higher than those for ^{14}C -cholesterol. Furthermore the rate of fall of the specific activity as indicated by the ratio of the 48 h to the peak specific ac-

tivity showed an inverse correlation to the pool size of serum free cholesterol in the case of ^{14}C ($r = 0.90$) but not in the case of ^3H ($r = -0.44$).

Role of serum α ketoisocaproate in synthesis of ^3H -cholesterol from ^3H leucine

The relatively low conversion of ^3H leucine to cholesterol as compared to that of ^{14}C mevalonate and almost tenfold variation from case to case could have been due to different dilution of the precursor into a large serum or tissue pool of leucine or its metabolites. Therefore α ketoisocaproate, the first metabolite during the conversion of leucine to HMG, was quantitated in serum. Table II shows that the values vary between 0.30 and 0.91 mg/100 ml. However there was no correlation to the respective peak specific activities of serum free ^3H -cholesterol suggesting that ^3H -cholesterol synthesis was not dependent on the α ketoisocaproate concentration. Further

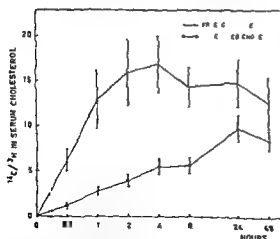


Fig 2 The ratio $^{14}\text{C}/^3\text{H}$ of both serum free and esterified cholesterol after simultaneous administration of DL mevalonate C and DL leucine-4,5 ^3H intravenously to human subjects

Table II Serum α ketoisocaproate concentration, serum triglyceride pool and conversion of DL leucine-4,5 ^3H into serum triglycerides in man

Case no	Serum α ketoisocaproate (mg/100 ml)	Serum triglyceride pool size (g ^a)	Serum ^3H triglycerides (of dose at peak SA ^b)
1	0.30	5.90	0.0873
2	0.39	7.03	0.0523
3	0.46	3.24	0.0302
4	0.66	3.06	0.0105
5	0.91	18.10	0.2170
6	0.64	5.23	0.0667
7	0.44	2.28	0.0101
Mean	0.57	6.36	0.0677

^a Plasma volume was assumed to be 4.5% of body weight.

^b SA = specific activity (dpm/mg)

Table III Turnover of serum esterified cholesterol in man

Case no	Serum esterified cholesterol		Turnover of ^{14}C -cholesterol		Turnover of ^3H cholesterol	
	(mg/100 ml)	Pool size (g^a)	(/hour)	(mg/hour)	(/hour)	(mg/hour)
1	439	11.50	1.060	122.0	1.030	118.0
2	409	11.80	0.617	72.7	0.483	56.9
3	406	6.67	0.907	60.5	2.000	133.0
4	333	10.20	0.595	60.6	0.769	78.4
5	363	13.20	0.866	114.0	1.470	188.0
6	189	6.51	1.490	97.0	2.150	143.0
7	177	5.26	0.825	43.4	1.190	62.6
Mean	302	9.29	0.909	81.4	1.300	111.0
$\pm \text{s.e.}$			± 0.115	± 11.2	± 0.240	± 18.3

^a Plasma volume was assumed to be 4.5 % of body weight

more no significant amount of radioactivity was found in a ketoisocaproate isolated from 1.5 ml of serum $2\frac{1}{2}$ h after administration of ^3H leucine. Thus its turnover rate must have been very rapid or its specific activity was already initially very low.

Incorporation of ^3H leucine to serum triglycerides

Because serum triglycerides are mostly synthesized in the liver the appearance of ^3H in this lipid class was measured in order to show whether ^3H leucine was converted to acetate in the liver. Table II demonstrates that the fraction of ^3H leucine incorporated into serum triglycerides at the peak specific activity was approximately the same as that found in serum free cholesterol. Further the larger the serum triglyceride pool the more ^3H triglycerides were found in the circulation ($r=0.96$). No association was found between the radioactivity in serum triglycerides and serum free cholesterol.

Turnover of serum esterified cholesterol

As presented in Fig. 2 the ratio $^{14}\text{C}/^3\text{H}$ in serum esterified cholesterol increases constantly but more slowly than in free cholesterol up to 24 h. Accordingly ^3H -cholesterol esters were released slightly faster than ^{14}C cholesterol esters into the circulation and in relation to ^{14}C cholesterol in higher concentrations than into free cholesterol.

This suggested that the turnover of ^3H esters could have been faster than that of ^{14}C esters. Therefore the turnover data were calculated for both ^3H and ^{14}C cholesterol esters. It will be seen from Table III that ^3H cholesterol esters actually turned over faster than ^{14}C esters in the two normocholesterolemic subjects and in three of five hypercholesterolemic. The turnover rate of ^3H -cholesterol esters correlated to that of ^{14}C cholesterol esters ($r=0.76$). However the calculated turnover rates as well as the fractional turnover rates were approximately the same in hypercholesterolemic and normocholesterolemic subjects and did not show any significant correlation to the radioactivities found in serum free cholesterol at the peak specific activity.

DISCUSSION

The fraction of injected ^3H leucine found in serum cholesterol in man was only about one hundredth of that of ^{14}C mevalonate. The latter if taken up by the cell is however incorporated preferentially to cholesterol. Leucine on the other hand could be incorporated into proteins or converted through several intermediary steps to HMG and further to ketone bodies and acetate (see Introduction). Thus the dilution of the radioactivity of leucine to its different metabolites before incorporation to cholesterol could have been markedly higher than that of ^{14}C mevalonate to its endogenous pool. Furthermore ^3H leucine is first diluted into serum leucine (concentration 1–2 mg/100 ml (21)) followed by dilution into the intracellular pool the concentration of which is 30–50 times that in serum (21). Serum and tissue levels of mevalonic acid are not known.

Since the liver at least in the rat has a low activity to metabolize leucine (mainly due to the small amount of leucine transaminase (11)) one may assume that the contribution of the liver to the cholesterol synthesis from leucine is negligible. From the extrahepatic organs the muscle tissue catabolizes leucine readily (20) and has in addition a large mass being thus able to convert leucine at a high rate to HMG and possibly also to cholesterol. It could be postulated that this cholesterol from muscle or from other extrahepatic organs may be the major source of serum cholesterol known in man to originate largely

from outside of the liver (3, 22, 23). The *in vivo* studies by Kabara have shown that leucine is converted to cholesterol with a relatively high specific activity in muscle of normal mice and in mice with muscular dystrophy (12). Our *in vitro* studies on rats demonstrated that cholesterol synthesis in muscle from leucine was higher than that from acetate or even from mevalonate. Further, the incorporation of this amino acid into serum cholesterol was reduced markedly less than that of acetate in rats whose hepatic cholesterol synthesis from acetate and HMG was blocked by cholesterol feeding (18).

The relatively high conversion of leucine to serum triglycerides in subjects of the present study indicates that this amino acid (or its metabolites, e.g. β -ketoisocaproate) was actually metabolized by the liver not only to HMG but also further to acetate and fatty acids. Thus the hepatic H-cholesterol may have been synthesized from ^3H HMG formed either directly during leucine catabolism or from ^3H acetate after the acetate pool was labelled by the breakdown of ^3H HMG. The extensive dilution of ^3H leucine and the apparent hepatic synthesis of ^3H cholesterol make it difficult to evaluate the quantitative role of leucine as the precursor of serum cholesterol in man and the contribution of extra-hepatic tissues to synthesis of serum cholesterol from leucine.

Although the amount of free ^3H cholesterol in serum and the turnover of serum esterified ^3H cholesterol after ^3H leucine was generally in accord with the respective ^{14}C values from ^{14}C mevalonate, some differences were found. The faster appearance of free ^3H -cholesterol in serum as compared to that of ^{14}C -cholesterol suggested that the intracellular localization of cholesterol synthesis from the two precursors and thus the release of resulting free ^3H and ^{14}C cholesterol into the circulation could have been partially different. More probably, however, the uptake and the subsequent conversion to cholesterol of leucine were faster than of mevalonate in one or several tissues. This early release seems not to be specific for leucine-cholesterol because by employing the simultaneous administration of ^3H mevalonate and ^{14}C acetate (15), ^{14}C -cholesterol appeared faster and remained longer in serum than ^3H -cholesterol resulting in a peak in the $^3\text{H}/^{14}\text{C}$ time curve (16) just as the ratio $^{14}\text{C}/^3\text{H}$

did in the present study. The fast labelling of serum free ^3H -cholesterol could have resulted in a faster esterification and higher turnover of ^3H cholesterol as compared to ^{14}C cholesterol. However, the radioactivity found in serum free cholesterol at the peak specific activity did not correlate to the turnover of the serum esterified cholesterol. Although the esterification is known to take place slowly also in plasma (7), Kabara (12, 13) observed that the specific activity of esterified tissue cholesterol was higher than that of free cholesterol within 15 min after administration to mice of either ^{14}C leucine or ^3H acetate. Cholesterol formed from these precursors had however a higher esterification than that formed from ^3H mevalonate. This could explain the fast appearance of esterified ^3H cholesterol in serum in the present study.

The amount of ^{14}C acetate or ^{14}C mevalonate appearing in plasma free cholesterol has been pointed out to correlate to the size of the plasma free cholesterol pool (10, 19). The results of the studies on ^{14}C cholesterol confirmed this finding in the present study, while the incorporation of leucine seemed to be less dependent on the pool size of serum free cholesterol. Furthermore, Hennes et al. (10) but not Nestel and Monger (19) found the reduced rate of fall in the specific activity of plasma free cholesterol during the first day in hypercholesterolemic subjects, a finding confirmed in the present study for ^{14}C but not for ^3H cholesterol. The significance of these findings remains uncertain. The relatively high amount of radioactivity appearing in serum free ^{14}C cholesterol pool of hypercholesterolemic subjects of the present study is hardly an indicator of the magnitude of endogenous cholesterol synthesis, mainly because the sterol balance, which in the steady state equals the endogenous cholesterol synthesis, appears to be normal in hypercholesterolemic subjects (17). The lack of correlation between the portion of the label transferred into the serum free cholesterol pool at the peak specific activity and the turnover of serum esterified cholesterol suggests that the former is not either primarily dependent on the esterification of serum free cholesterol. Assuming that the equilibration of newly formed serum free cholesterol to other free cholesterol pools proceeds at a constant rate independently of the pool size, the amount of label found in plasma free cholesterol

is high in hypercholesterolemia while the rate of fall of the specific activity is low

The fractional turnover rate of cholesterol esters that tended to be higher for ^3H than for ^{14}C cholesterol was somewhat lower than reported by Nestel and Monger (19). Even then however the daily average turnovers of ^3H cholesterol and ^{14}C cholesterol were about 2.7 and 2.0 g respectively. In the steady state this amount must also be removed. The removal probably involves mainly the hydrolysis of the esters back to free cholesterol (8) at least it cannot be exclusively due to transfer of the esters into faeces by the liver and intestine because the daily faecal excretion of cholesterol and bile acids usually appears to be less than 1 g (17).

ACKNOWLEDGEMENTS

This investigation has been supported by grants from the State Medical Research Council of Finland, Sigrid Jusélius Stiftelse and US (no 5 RO TW00218) Public Health Service. The National Institutes of Health.

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KÖHLMEIER DEGOS DISEASE (MALIGNANT ATROPHIC PAPULOSIS)

Report of the First Scandinavian Case

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Abstract A report is given of the first Scandinavian case of malignant atrophic papulosis (Kohlmeier Degos disease) in a man aged 31. The patient presented the typical morphological changes in the skin and intestine. This case is the first in which similar vascular lesions have been demonstrated in the lungs, liver and pancreas.

In 1941 Kohlmeier (4) described a remarkable cutaneo-intestinal syndrome in a 21 year old man and assumed the findings to be manifestations of thrombo-angitis obliterans. In the following year Degos et al (2) reported a similar case and thought the syndrome to be a well defined entity which they first called papulo-squamous dermatitis but afterwards malignant atrophic papulosis to indicate the poor prognosis of the condition.

A further 19 cases have since been published. The literature has been reviewed by Sidi et al (7) and by Strole et al (8).

The case reported below is the first known instance of this rare disease in Scandinavia. It is also the first case in which typical vascular lesions of Kohlmeier-Degos disease have ever been found in the lungs, liver and pancreas. The diagnosis was made at histological examination post mortem and historical data, particularly those relevant to the evolution of the cutaneous lesions, were far from complete.

CASE REPORT

A man aged 30 had mild pneumonia in 196 and in 1963. In the summer of 1965 he had a rash on the trunk and thighs. Later (after the patient had died) the patient's wife described these lesions as red patches which afterwards became pale, first centrally and then disappeared. That year he also had diffuse epigastric pain but roentgenography revealed nothing remarkable. In August 1966 the symptoms increased with frequent vomiting and considerable loss of body weight (0 kg within two

months). He was admitted to his local hospital for investigation. A few days after admission he had perforating ulcer in the anterior part of the stomach for which he was operated upon in October 1966. Careful abdominal palpation and examination revealed nothing remarkable apart from slight enlargement of the spleen. Chest X ray after the operation showed diffuse coalescent densities on both sides (no abnormalities found at chest X ray in 1964). He was transferred to the Chest Clinic Boden for further investigation. He had fever (38-39°C) for a week and on December 4, 1966 an acute attack of abdominal pain suggesting perforation. Surgical exploration revealed perforation of the ileum with considerable local changes for which the intestine was resected and anastomosed end-to-end. He was then transferred to the department for intensive therapy.

On admission to that department a careful inquiry was again made into the patient's history, this time including also a search for signs of intoxication and allergy but revealed nothing of interest. The patient was very cachectic but otherwise in a good general condition. Chest X ray showed further progression of the densities in both lungs. The densities were partly coalescent but here and there they were rounded and well defined and appeared malignant. ESR 30 mm/h. Hb 11.1 g/100 ml. RBC 4 mill. Hct 38% platelet count 72,000. WBC 8300 with 76% neutrophils. Serum electrolytes normal. Serum protein 4.4 g/100 ml. Electrophoresis: albumin 44%, alpha-globulin 10.8%, alpha₂-globulin 14.9%, beta-globulin 15.9% and gamma globulin 14.1%. Plasma creatinine 0.7 mg/100 ml. Urine: salts specific gravity and osmolality normal. Urine sediment: -3 red blood cells, 5-10 white blood cells, only few bacteria. Sternal puncture markedly increased myelopoiesis with shift to the left and dominated by myelocytes. Increased number of mature plasma cells. Reticulum markedly increased. Increased alkaline phosphatase activity of neutrophils.

Culture of throat swabs, bronchial secretion, urine and blood was repeatedly negative. Repeated examination for mycobacteria with macroscopic examination, culture and guinea pig inoculation negative. Culture and agglutination for pseudotuberculosis negative. Antistreptolysin titre 70 units per ml, antistaphylococcal titre 0.36 unit per ml. No agglutination of *Salmonella* O-antigen H-antigen Brucella abortus or *Tularense*. Bronchoscopy with biopsy and

ciological examination revealed nothing remarkable.

Because of his cachectic condition the patient was given adequate nutrition parenterally. Tuberculosis was strongly suspected and treatment with PAS, INH and streptomycin was started on December 7. Fever persisted (38–39°C) and cardiac frequency was high (170–130 ECG normal except for tachycardia). On December 10 the body temperature rose still more (41°C) and a chest X-ray showed progression of the changes besides which the liver was palpably enlarged. PAS was therefore withdrawn. On December 14 he again had peritonitis and was again subjected to surgical exploration. No effusion was found but the small bowel showed marked changes with fibrin-coated lesions and a considerable amount of whitish round infiltrates with palpable thickening of the serosa. A small part of the omentum was excised. The patient tolerated the operation fairly well. Microscopic examination of the material obtained at the operations yielded no information of diagnostic value.

The operative specimens were roughly 10 cm long segments of the ileum with diffuse thickening of the wall and numerous pale gray to yellow irregular plaques on the serosal surface. Centrally there was a perforation, the size of a peppercorn and an adjacent circular stricture. The muscular fold were excised.

Microscopically the perforation was lined by thick fibrous necrotic masses and bordered by granulation tissue with abundant inflammatory cells mainly granulocytes. Adjacent submucosa was oedematous with dilated vessels and mild fibrosis. The middle-sized arteries showed subendothelial fibrosis with marked narrowing of the lumina. Some areas of the serosal surface were covered by a thin fibrin coat with an admixture of granulocytes and single fungi of the species *Candida albicans*.

The pathological diagnosis was infarction of the ileum of obscure origin with perforation and peritonitis. In retrospect, the lesions were identical with those described and illustrated under necropsy findings.

One week after the operation the patient had chills and intense parenteral treatment with penicillin (4 million units per hour) was tried with simultaneous steroid therapy to suppress the pulmonary reaction. For a few days the fever disappeared, but tachycardia persisted. On December 28 he had symptoms of a new perforation. Exploratory surgery revealed a palm-sized perforation of the stomach and severe peritonitis. The lesion was closed with a fairly satisfactory result. The patient made a good recovery but had high grade fever and tachycardia. At operation tracheotomy was done and even after the operation the patient could not breathe without a respirator. As before, new laboratory and bacteriological tests proved negative. The serum bilirubin and transaminases gradually increased and the patient developed a bleeding tendency with an Ivy bleeding time of more than 36 min. The plastic clotting time was normal. Fibrinolytic activity with fibrinolysis 0.2 mg/ml/h and fibrinogenolysis 0.31 mg/ml/h, fibrinogen 0.15 g/100 ml. He was given transfusions of fresh blood and EACA, which resulted in normalisation of these values and his bleeding time. On January 1, 1967, treatment with erythromycin was tried because of a period of high grade fever and further

progression of the pulmonary changes. The response was favourable: the fever disappeared and for the first time the pulse rate became normal and the patient's general condition improved. Within a few days the pulmonary changes regressed considerably. After four days the serum bilirubin began to rise rapidly, the liver increased further in size, tachycardia returned and body temperature rose. Erythromycin was withdrawn and other antibiotics were tried but without effect. On January 9 he was treated with hypothermia with fair success until January 13 when the patient went into irreversible shock and died on January 16.

Necropsy findings

Complete necropsy including the brain, was performed at Centrallasarettet, Boden. The external examination revealed jaundice and generalised petechial cutaneous haemorrhages. The chief finding was fibrinopurulent peritonitis with widespread adhesions and a right-sided subdiaphragmatic abscess. The gastrointestinal tract was dilated and the content heavily bloodstained. The intestinal serosa was covered by thick patches of pus and the intestinal wall was oedematous. The mucosa of the stomach and small intestine was thickened, oedematous and showed widespread haemorrhages. The middle of the dorsal wall of the stomach had a 1.5 × 1.5 cm perforation coalesced with the left liver lobe.

The mesenteric lymph nodes were enlarged. The mesenteric vessels were dissected but no nodules, thrombi or other gross changes were found.

The liver showed well-defined haemorrhagic infarcts. No gross changes were seen in the large trunks of the hepatic artery or portal vein.

Other autopsy findings were scattered small necroses in pancreas, tracheobronchitis and multiple haemorrhagic pulmonary infarctions up to the size of almonds. No thrombi were seen in the main branches of the pulmonary artery.

Microscopic study of the operative specimens and specimens obtained at autopsy showed largely uniform pathological appearance of affected vessels in the gastrointestinal tract, liver, pancreas and lungs. The typical picture consisted of intravascular endothelial proliferation and progressive occlusive fibrosis in small and medium-sized arteries (Figs 1–4) with or without secondary thrombosis and consequent ischaemic infarction.

The lesions in the gastrointestinal tract, from the stomach through colon, were of uniform appearance and involved all the layers of the intestinal wall. The submucosa was affected most with severe congestion and oedema. The blood and lymph vessels were dilated and often surrounded by fibrous tissue. The arterial walls were irregularly thickened, hyalinised and encircled by a few lymphocytes. The disorganisation was due to an eccentric subendothelial fibrosis with preserved elastic interna leading to occlusion of small vessels and to reduction in the size of the lumina of larger vessels. Thrombi of various ages and sizes were adherent to the vessel walls. Veins were also affected though to a lesser degree. Occasionally though rarely a few areas of active vasculitis were recognized with involvement of the vessel wall by lymphocytes, histiocytes and some neutrophils.



Fig 1 Submucosal artery in ileum (operative specimen) with marked subendothelial fibrosis. Haematoxylin-eosin $\times 100$

Fig 2 Same section as in Fig 1. Internal elastic lamella intact. van Gieson-elastica $\times 100$

Fig 3 Submucosal artery in ileum. Marked subendothelial fibrosis and mild lymphocyte reaction in surrounding tissue. Haematoxylin-eosin $\times 100$

Fig 4 Same section as in Fig 3. Internal elastic lamella intact. van Gieson-elastica $\times 100$



Fig 5 Pulmonary infarction with two small arteries almost occluded by loose subendothelial fibrosis van Gieson-elastica $\times 85$

The mucosa showed patches of necrosis with ulcerations and splitting of the musculi mucosae. In severely affected areas and around the large perforation in the stomach all layers of the wall were infarcted and showed signs of superimposed infection with abundant inflammatory cells mainly neutrophilic leucocytes and some fungi of the species *Candida albicans*.

Other segments of the intestine showed vascular changes also in muscularis propria and some fibrous tissue was deposited in both muscle layers. There was abundant fibrinopurulent exudate on the serosal surface and in the subserosal connective tissue.

The mesenteric lymph nodes showed non specific inflammation and no vascular changes.

Lesions identical with those in the gastrointestinal tract were seen in small blood vessels in the lungs (Fig 5) liver and pancreas (Fig. 6). The liver and pancreas showed widespread haemorrhagic necrosis with well defined leucocytic margins. The liver surface was covered with thick patchy layers of fibrin containing numerous granulocytes. Also the infarcted pulmonary parenchyma showed abundant granulocytes and the adjacent lung tissue was covered by haemorrhagic pneumonia without any histologically demonstrable microorganisms. The visceral pleura was fibrously thickened and its surface was partly covered by fibrin swarming with leucocytes.

No morphological changes were found in the brain.

DISCUSSION

Kohlmeier Degos' disease papulosis atrophicans maligna is a remarkable systemic disorder of the vessels. It is characterised by practically pathognomonic skin lesions. The gastrointestinal tract is the most often affected of the organ systems involved and the outcome is generally fatal owing to multiple intestinal infarctions with perforations and consequent fulminating peritonitis. The cause of the disorder is obscure and no effective treatment is yet available. Clinically the disease is readily distinguished from polyarteritis nodosa and thromboangiitis obliterans and from a pathological point of view it cannot be regarded as a manifestation of these diseases.

The cases on record were seen in 17 men and two women. The commonest age of onset is 15 to 25 years. The disorder is usually fatal within one year of the appearance of the skin lesions.

The initial skin lesions which are scattered over the trunk, neck and proximal portions of the limbs develop slowly. The lesions are asymptomatic.



Fig 6 Eccentric subendothelial fibrosis of medium sized artery in pancreas. Adjacent parenchyma is partly necrotic and shows scattered round cells van Gieson-elastica $\times 85$

matic pink to red rounded or oval papules. Within a few days they become umbilicated and there appears a porcelain white atrophic centre encircled by a slightly elevated erythematous telangiectatic border. Some weeks later the lesions begin to disappear and leave behind atrophic white spots but lesions in different stages of development can always be found.

Histopathologically the picture of the skin eruptions is that of necrosis in dermis and subcutaneous tissue with underlying oedema and a non specific inflammatory reaction which is followed by sclerotic atrophy in the dermis and atrophy and hyperkeratosis of the epithelium. These lesions are the result of focal changes of small and medium sized arteries in the subepidermal tissues with progressive subendothelial occlusive fibrosis with consequent ischaemic infarcts. Veins and lymphatics are affected later by peripheral fibrosis. The skin and small bowel are the commonest sites of the spotty vascular changes and the abdominal symptoms are as a rule ushered in by the skin lesions. The onset of the abdominal symptoms generally marks the beginning of steady deterioration with a fatal issue. As long as the bowel is only slightly affected the patient generally feels well except for mild abdominal symptoms and fair loss of weight. Progression of the intestinal lesions is accompanied by ileus, nausea, haematemesis and melena but an acute attack of intense epigastric pain may be a sign of the initial stage of the abdominal syndrome.

Morphologically the intestinal lesions do not differ from the cutaneous lesions but the vasculitis, thrombosis and adjacent inflammation are often more prominent in the intestine than in the skin.

Contrary to what was supposed on the basis of the first cases published the disease is not confined to the skin and/or intestines. Thus identical focal vascular changes damaging the tissue have been described in the brain, kidney, heart, eyes, mesentery and bladder (1, 3, 5, 6, 7, 8, 9). This paper is the first to report similar vascular changes in the lungs, liver and pancreas. Such lesions may perhaps be found also in other organs and possibly early enough to provide a clue to their aetiology.

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THE PROPHYLACTIC ANTIARRHYTHMIC EFFECT OF QUINIDINE IN MYOCARDIAL INFARCTION

A Controlled Clinical Trial

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Abstract A controlled study has been carried out to examine the possible prophylactic antiarrhythmic effect of quinidine sulphate 0.2 g t.i.d. in acute myocardial infarction. No difference was found in the occurrence of the various types of arrhythmias apart from extrasystoles, which were significantly less frequent in the treated than in the non-treated group. An analysis of the causes of death revealed no difference in sudden and unexpected deaths between the two groups. There was rather a vague impression that quinidine might have contributed to dangerous complications and death in a few patients. The possible reasons for these findings are discussed in relation to the observations from the literature.

Rates of mortality and statements of death mechanisms in acute myocardial infarction vary considerably in the different series published. The mechanisms of death are usually put down as arrhythmias, cardiac failure and cardiogenic shock. In many cases it is difficult to decide what is the main and what is the secondary cause. Mower et al. (11) attribute 50% of the deaths to "rhythm disturbances", 30-35% to heart failure and the rest to cardiogenic shock. Hershman and Turtel (6) claim that 40 to 50% of the deaths due to myocardial infarction are caused by "electrical failure" while the rest are due to "mechanical failure". In other words about one half of all deaths due to myocardial infarction seem to occur from disturbances of normal impulse formation and conduction. Many of these cases suffer a "sudden and unexpected death" and undoubtedly some of these patients may have "hearts too good to die". If a further reduction in mortality is to be obtained it seems rational to concentrate on this category of patients. Much less is to be expected from the treatment of patients with

mechanical failure due to severe myocardial damage caused by the infarction.

The prophylactic use of antiarrhythmics in acute myocardial infarction has been scarcely dealt with in the literature. The results of the few properly conducted examinations vary so much that it is difficult to draw any definite conclusions.

Cutts and Rapoport (4) giving up to 0.9 g quinidine t.i.d. found no effect upon mortality and frequency of arrhythmias heart failure or shock.

Boone and Papavas (2) found a highly significant improvement of the mortality rate in a group treated with quinidine compared with a control group. Their work, however, is retrospective and the groups are heterogeneous (differences with respect to anticonvulsant therapy) and numerically very different.

Scherf (13) proposes that all patients with acute myocardial infarctions routinely should have quinidine therapy when contraindications exist.

Berg (1) and Revell (12) found no effect on mortality rate when giving quinidine and procainamide respectively. The effect on the arrhythmias was uncertain and inconclusive.

Huht et al. (9) report no effect of quinidine sulphate 0.2 g t.i.d. on mortality rate. Nor do they find any prophylactic action against arrhythmias. They also state that quinidine may be harmful on account of the depressive action on impulse formation and conduction. Their quinidine group and control group, however, are from different periods of time.

Table I Composition of material

Age distribution	Quinidine group (94)		Control group (107)	
	♀	♂	♀	♂
<50	0	6	1	12
50-59	3	14	4	11
60-69	9	22	6	33
70-79	15	18	10	16
>80	5	2	0	7
Total	32	62	21	86
Average age	71.5	63.4	63.9	63.3
Range	51-84	42-81	46-79	36-89

With reference to the discrepancy between the conclusions in the different series published the prophylactic antiarrhythmic effect of quinidine has been studied in a controlled clinical trial

MATERIAL AND METHODS

All patients with acute myocardial infarction and a case history shorter than 48 hours before admission to the hospital were included during the period from September 1 1964 to August 31 1966. All the patients who died within the first 1.4 hours were excluded from the material. Ninety-four patients arriving on odd days of the month were given quinidine sulphate 0.2 g three times a day. One hundred and seven patients arriving on even days as controls. No patient has been included more than once.

The plan was to try to answer the following questions:

- 1 Does quinidine 0.2 g three times daily provide any prophylactic effect against (a) Sudden cardiac death? (b) Arrhythmias (some of which might be potentially fatal)?
- 2 Are signs of myocardial depression recorded due to the above mentioned dose of quinidine?
- 3 Does quinidine 0.2 g three times daily cause dangerous or fatal arrhythmias or blocks?
- 4 How often do side-effects necessitate withdrawal of quinidine in an unselected group of patients with acute myocardial infarction?

A relatively small dose of quinidine was chosen because larger doses routinely might produce more toxic reactions.

Cases with atypical ECG (about one third of the material) were included provided that the case history and biochemical findings made the diagnosis certain.

The patients were examined at least twice daily. Special attention was paid to auscultation of the heart notes being made about gallop rhythm, friction rubs, arrhythmias, signs of cardiac failure and thromboembolism. We were especially aware of side-effects of quinidine.

Table II Complicating diseases

	Quinidine group		Control group	
	♀	♂	♀	♂
Hypertension	14	22	10	28
Diabetes	9	5	3	5
Previous apoplexia	0	3	0	6
Rheumatic heart disease	6	1	2	0
Previous angina pectoris	19	29	13	34
Previous infarction	8	8	4	15
At least one complicating disease (except angina pectoris and/or infarction)	49		40	
Two or more complicating diseases	12		5	
Shock on admission	20		31	
Median SGO T value (Sigma units)	118	180	150	156

Table III Localisation of infarctions

	Quinidine group		Control group	
	♀	♂	♀	♂
Anterior	9	25	10	22
Posterior	13	15	6	33
Anterior + posterior	11	11	0	2
Atypical ECG	10	22	5	29

Table IV Digitalis treatment started during hospitalisation

Indications	Quinidine group	Control group
Left ventricular failure	20 (2†)	25 (4†)
Right ventricular failure	0	0
Combined failure	5 (1†)	4 (1†)
Arrhythmias	5 (0†)	4 (1†)
Arrhythmias + failure	4 (1†)	1 (0†)
Shock	2 (1†)	5 (3†)
Shock + failure	4 (1†)	5 (3†)
Cardiomegaly (without obvious failure)	0	7 (0†)
Total	40 (6†)	46 (14†)
Previously treated with digitalis	14 (4†)	13 (2†)
Total number of cases with previous or present digitalis treatment	54 (10†)	56 (16†)
Left hospital on digitalis treatment	44	43

Table V Arrhythmias at hospital (arrhythmias during first 24 h not included)

Type of arrhythmia	Quinidine group	Control group
Extrasystoles	49	58
Atrial fibrillation	13	15
Atrial flutter	4	3
Sinus tachycardia	19	17
Sinus bradycardia	9	13
Nodal rhythm	3	4
Nodal tachycardia	1	1
AV block (Grade I-III)	10	12
Ventricular tachycardia	2	2
Ventricular fibrillation	1	1
Patients with one or more of the above mentioned disturbances of rhythm and/or conduction	■	79
Patients without any recorded arrhythmia or conduction disturbance	25	28

All patients were treated with anticoagulants (phenyl indanedione). An attempt was made to keep the thrombotic test value between 10 and 20. They were early out of bed i.e. when temperature signs of shock or heart failure did not contraindicate (in most patients within one week). Patients with uncomplicated infarctions usually left the hospital within three weeks.

Table VI Survey of death rates

	Quinidine group	Control group
Arrived in hospital within 48 h from onset of symptoms	103 (67 o)	112 (91 o)
Died during the first 12 h in hospital	9 (5 d)	5 (5 o)
Left hospital alive	111 (58 d)	89 (72 d)
Total mortality	$\frac{18}{103}$ 100 = 17.5	$\frac{23}{112}$ 100 = 20.5
Mortality during observation period	$\frac{9}{94}$ 100 = 9.6	$\frac{18}{107}$ 100 = 16.8
Male mortality during observation period	$\frac{4}{62}$ 100 = 6.5	$\frac{14}{86}$ 100 = 16.3
Female mortality during observation period	$\frac{5}{32}$ 100 = 15.6	$\frac{4}{21}$ 100 = 19.0

The two groups are fairly comparable as a whole with regard to age distribution, complicating diseases, presence of shock and values of SGO T (Tables I and II). Further more no significant difference was observed between the groups concerning the localisation of the infarctions or digitalis treatment during hospitalization (Table III and IV).

Table VII Survey of deaths in quinidine group

I = Previous infarction | A p = angina pectoris | A = anterior infarction | P = posterior infarction | Atyp = atypical ECG

Initials	Age	Sex	Complicating heart disease	Type of infarct	Quinidine side-effects	Causes of death		Time of death (d)
						Primary	Secondary	
J A	78	♀	Aortic stenosis Aortic insuff 12 years ago A p 5 y	A		Shock		1½
A H	72	♀	A p 5 y	■		Mors subita		4
O H	66	o	11½ y	A	Asibenzia Anorexia	Shock (re inf)	Heart failure	21
E J	79	♀	Aortic stenosis Mitral insuff 11 year ago A p 4 y	Atyp		Ventric fibrill		25
T L	65	♂	A p 6 y	■		Mors subita	Pre shock	8
G R	74	♂		A		Mors subita	Heart failure	19
A U	64	♂	12 years ago A p 2 y	Atyp		Shock	Heart failure Nodal tachyc	5
L O	■	♀		A	AV block (grade 3)	Shock	Heart failure	2
A Å	76	♀	A p 2 y	P	AV block (grade 3)	Shock	Heart failure Embolus of left fem artery	5

Five o average age 77.5 y

Four o average age 67.3 y

Table I The calculated number of human *Taenia* infections in millions

Geographical area	<i>Taenia solium</i>	<i>Taenia saginata</i>
North America	<0.1	21.5
Middle and South America	<0.1	1.3
Africa	0.5	0.2
Europe without USSR	<0.1	3.9
USSR in Europe	0.5	1.3
USSR in Asia	0.3	—
Asia excluding USSR	1.2	<0.1
Oceania	<0.1	<0.1

CASE REPORT

Mechanic aged 56 married right handed. Admitted December 1965. Nearly seven months history of periods lasting about ten seconds of vertigo feeling of unreality slight nausea and inadequate speech. The symptoms appeared from two to three times a week. Moreover the patient felt that his memory had deteriorated. Past history was noncontributory. Physical examination showed nothing remarkable. Laboratory findings were normal but ESR was 20 mm. EEG showed a moderate focal abnormality partly episodic but otherwise unspecific maximum in the left frontotemporal area, like temporal lobe epilepsy. Conventional films of the skull showed a calcified corpus pineale and small calcifications of the ependyma and pneumoencephalography showed a downward convexity in the roof of the left lateral ventricle. An angiography in the left common carotid artery (completed in the right side) revealed a Parasagittally situated poorly vascularized expansive lesion in the middle and back of the parietal area which was the size of a 1.5 egg. The patient was referred to the Neurosurgical Clinic at Karolinska Sjukhuset where in March 1966 an extracerebral irregular and in some places lobulated cyst was removed. It contained a green whitish milky fluid. The cyst was situated in the middle third of the venous sinus. Histologic diagnosis showed a colloid cyst without malignant features. In the postoperative course the patient had epileptic seizures of a generalized character which were alleviated by the administration of antiepileptic drugs. The patient's only complaint was an unpleasant olfactory sensation with almost complete anosmia. An initial hemiparesis of the right side regressed completely. A feeling of weakness in the right arm and leg could not be verified objectively. Laboratory studies were normal.

The patient lived at home for three months and was given antiepileptics. He suffered from one or two recurrent epileptic seizures every month he was unconscious for five minutes but without involuntary urination.

In the middle of July 1966 a right sided hemiparesis developed in the course of four days. On admission the patient could walk by himself. There was weakness in his right arm and leg, and slightly increased tendon reflexes on this side. Lumbar puncture showed normal cerebrospinal fluid pressure and a normal Queckenstedt's sign.

Lumbar fluid was clear colourless without increased cell count and protein 42 mg. The Mastix reaction was normal. The hemiparesis progressed and therefore in August 1966 the patient was again referred to the Neurosurgical Clinic at Karolinska Sjukhuset. The EEG then showed distinct deterioration compared with his condition in April 1966. An angiography in the left common carotid artery revealed a large nonvascularized area in the left parietal region. At operation no recidivation of the former cyst was observed but numerous small cysts containing clear fluid bulged from the subarachnoid space. From the cortical surface towards the dura and the falx a large number of arachnoid chords were seen, with a yellowish coating. The vasculature of the cortical surface was increased in the same way as in a chronic inflammatory state. Some yellow whitish rounded corpuscles each a large grain of rice were found in the arachnoida toward the falx. Histologic diagnosis of the extirpated tissue revealed a cysticercosis. In the postoperative course paresis of the right leg diminished while that of the right arm was almost complete. Owing to the diagnosis of cysticercosis repeated examinations for ova in the feces were made but with no result. Radiologic investigations of the lungs and the muscles of the femora did not show any abnormal findings. The total eosinophilic leucocyte count was normal as were subsequently repeated differential counts. Since his discharge in November 1966 the patient has lived at home taking antiepileptics and going in for physical training. Paresis has regressed to some extent and a year after discharge the patient is able to take long walks without support. He can raise his arm up to the level of his shoulder. The olfactory sensation is unchanged. The patient has suffered from five minor epileptic seizures at night. These were of short duration and without involuntary urination. EEG is essentially unchanged.

The patient was brought up on a farm where among other animals pigs were bred. From 1930 to 1950 he worked as a groom and then as a mechanic. However each year from about 1936 to 1955 he has bred four domestic pigs for slaughtering and use in the household. This has been done without meat inspection. He has not travelled abroad which could have involved a risk of exposure to taenia. There is no history of disease caused by worms except an otyunasis infection in childhood.

DISCUSSION

Extensive reports from different parts of the world show that there is relative agreement in the age distribution of patients with cysticercosis cerebri. The maximum age when the affection is contracted is between 20 and 40 and up to 50 years. Initially the parasites cause only minor disturbances and the patient can live for years with mild symptoms even with numerous cystic lesions in both hemispheres. Most often the cysticerci are found in the subarachnoid space in the basal nuclei in the ventricles and sometimes in

an intracerebral location. According to the symptomatology, different classifications have been introduced (8, 9).

Throughout the most frequent form seems to be the convulsive type of symptoms with initial symptoms of focal origin irrespective of whether their form is Jacksonian or temporal lobe epileptic. In a large percentage of these cases the EEG shows an active cerebral focus. The symptoms in the same patient vary widely during the course of the disease with mental disturbances and generalized epilepsy. At a multiple localisation there may be signs of increased intracranial pressure and cerebral edema. The cerebrospinal fluid may show eosinophilia, hypercytosis, slightly increased protein content with qualitative changes such as a raised gammaglobulin fraction. A positive reaction of complement fixation in a satisfactory titer for diagnosis exists in about 70%.

The hypertensive type of symptoms with headache, vomiting and papilloedema may mainly depend not only on a basal localization of the parasite with basal chronic leptomeningitis as a consequence but also on engagement of the fourth ventricle or fossa posterior. Especially when localization is in the fourth ventricle there will be intense symptoms owing to a total or subtotal blockade of the circulation of the cerebrospinal fluid from membranous formations which also surround the parasite.

The diagnosis is much facilitated if the patient has subcutaneous noduli accessible to biopsy or muscular or intracranial calcifications visualized by roentgenography. Arseni and Samitca (1) found that in about 90% of 65 cases of cysticercosis cerebri the cysticerci were localized solely in the brain. Only 10% of the cases with radiologically demonstrable muscular calcifications had intracranial calcifications which by no means exclude cerebral cysticercosis. There are few reports in the literature dealing only with the roentgenologic aspects of the condition. In an investigation of this kind Dorfsman (3) showed the presence of intracranial calcifications in four out of 126 cases verified at operation or post mortem. On the other hand the percentage of intracranial calcifications in clinically diagnosed cases of cysticercosis cerebri is said to be as high as 15%.

The classification of cysticercosis cerebri proposed by Stepień (9) and based on the clinical course in cases resembling 1. tumor, 2. cerebral

edema and 3. hydrocephalus is convenient since this classification reflects the prognosis as in cases of established cerebral cysticercosis therapy is only palliative. Stepień reported on 94 operated patients out of 132 and recorded an improvement or recovery in 75% of the cases in group 1, 53% in group 2 and only 28% in group 3. Our own case belongs to group 1 in this classification and consequently has a good prognosis.

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A SEVERE HAEMORRHAGIC DISORDER WITH PROLONGED BLEEDING TIME DUE TO A PLASMA DEFECT BUT WITH NORMAL FACTOR VIII

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Abstract A young girl had bleeding symptoms resembling those of severe von Willebrand's disease. At 12 years of age she had perious menorrhagia. The Duke bleeding time usually exceeded 30 min but all coagulation factors were normal and unlike what is seen in von Willebrand's disease factor VIII was normal. There was no pathologic fibrinolysis. The platelet adhesiveness was near the low limit of the normal range when tested with Hellem's whole blood method, and normal when examined with his plasma ADP method, but decreased when assessed with Salzman's method. The platelets appeared normal and aggregated and adhered normally to a glass slide. Aggregation after addition of ADP or connective tissue suspension was also normal. The prothrombin consumption test was usually normal. Fraction I-0 fresh plasma, and stored plasma shortened the bleeding time. There was no family history of a bleeding disorder and her parents showed no evidence of such a condition.

The findings suggest a special bleeding disorder of a new type resembling severe von Willebrand's disease but distinctly different from it. We have provisionally called the condition morbus Rita.

Congenital haemorrhagic disorders with prolonged bleeding time but normal platelet count may be divided into two main groups: one with primary platelet disorders such as thrombasthenia, and the other with primary plasma defects such as von Willebrand's disease.

Von Willebrand's disease is characterized by a dominant heredity, a prolonged bleeding time and a low factor VIII (20). Platelet adhesiveness as tested with Hellem's methods is normal (11) but mostly low when tested with Salzman's method (9-11). Platelet function is otherwise normal. The prolonged bleeding time can be shortened by administration of fraction I-0 or fresh plasma.

Von Willebrand's disease is classified as severe when the Duke bleeding time exceeds 20 minutes otherwise as mild. In the severe form factor VIII

is as low as 1-20% in the mild form 20-60 (20).

In Sweden von Willebrand's disease has been diagnosed in more than 200 members of 100 families. Patients with primary platelet defects of varying severity have also been observed (9-10, 12). In addition one patient had a severe bleeding disorder with a markedly prolonged bleeding time but differing in many respects from other known bleeding disorders. Factor VIII and other coagulation factors were normal and the defect was corrected by plasma not necessarily fresh. Since the patient may have some hitherto unknown type of congenital haemorrhagic disorder the case is reported in detail.

MATERIAL AND METHODS

Human fraction I-0 containing AHF (factor VIII) was prepared at Chemistry Department II Karolinska Institute, Stockholm, Sweden by the glycine method of Blomback and Blomback (4). One dose of fraction I-0 is prepared from 1400 to 1600 ml of fresh normal plasma and contains about 3 g of protein. Usually one half of one dose of fraction I-0 was given on each occasion. Half a dose of fraction I-0 dissolved in 100 ml of isotonic saline has an AHF activity 5 to 8 times that of 100 ml fresh normal plasma.

Collect on of blood was performed in the way described earlier (11).

Coagulation tests All the methods used for preparing the blood samples and for determining the various coagulation factors have been described elsewhere (23). The factor VIII activity of plasma was assessed from its normalizing effect on the recalcification time of haemophilic A plasma (20, 23-25) and the amount of AHF (factor VIII) present was expressed as a percentage of that found in a normal standard consisting of pooled plasma from ten normal individuals.

The *bleeding time* was mostly determined with Duke's method, using standardized haemolets (Dade Reagent, Inc.,

Miami Florida USA) Determinations were performed on both ears Normal range 1 to 4 mm Use was also made of the method of Ivy as modified by Borchgrevink and Waaler (5 11 25)

The fibrinolytic system was studied in the way described previously (26 27)

Platelet counts were made according to Hellem's modification (16) of Nygaard's method (29) Control counts were also made by the method of Björkman (2)

Platelet suspensions were prepared and tested for platelet factors 1 3 and 4 as described previously (28)

Platelet rich plasma was prepared by centrifuging citrated blood (1 part 3% sodium citrate dihydrate and 9 parts blood) drawn by the silicon technique at 185 mm for 10 min immediately after collection

Platelet deficient plasma was prepared by centrifuging platelet rich citrated plasma at 16 000 g for 30 min in plastic tubes at +4°C and then carefully pipetting off the supernatant plasma without disturbing the layer of platelets

Platelet adhesiveness was measured according to a slight modification (11) of Hellem's whole blood method (16) According to this method citrated whole blood is passed through a column of glass beads and the percentage of adherent platelets is calculated Platelet adhesiveness in platelet rich plasma after addition of ADP in various concentrations was determined according to a slight modification (11) of the method of Hellem et al (17) Platelet adhesiveness was also measured with the original method of Salzman (31) according to which whole blood is rapidly passed directly from the vein through a glass filter

Platelet adhesion aggregation and spreading were studied by phase contrast microscopy of a drop of platelet rich citrated plasma placed on a glass slide under a cover glass Both ordinary and siliconized glass slides were used Platelet spreading and aggregation were also investigated by Dr J Suharrer a member of Dr Breddin's staff using Breddin's original methods (6 7)

Platelet aggregation after addition of connective tissue suspension or ADP was studied macroscopically microscopically and with the aid of a photometer (9 12)

Clot retraction was determined in diluted platelet rich plasma according to a modification of the Voss method (12)

The tourniquet test was performed by placing a blood pressure cuff around the upper arm and inflating it to a pressure intermediate between the systolic and diastolic pressure The number of punctiform haemorrhages that had appeared within 10 min in an area of 20 cm² was counted A count of more than 10 was considered positive

CASE REPORT

The patient was a girl born in 1950 Since early childhood she had had an increased bleeding tendency and had bruised readily She had never had gingival bleeding and bled profusely after cuts On several occasions she had had epistaxis requiring cauterization At the age of 10 she was admitted to hospital because of severe bleeding after extraction of a tooth The bleeding time was prolonged despite a normal number of platelets She was then referred to our laboratory for investigation At one

determination of the bleeding time the patient bled profusely from the wound for several hours Owing to her severe bleeding tendency severe menstrual bleedings were expected and she was therefore hospitalized at the onset of her first menstruation which occurred when she was 12 years old She then bled profusely for 14 days and the haemoglobin fell from 13.8 g/100 ml to 7.3 g/100 ml She received 3 pints of blood In the following years menorrhagia was very troublesome and on five occasions shock supervened In December 1963 the menstrual flow continued for 14 days She then left hospital but was readmitted some hours later in a state of shock owing to recurrent severe bleeding This recurrence lasted for 3 weeks during which she received altogether 5 pints of blood In July 1964 the flow lasted for more than a month and in May June and August 1966 for 2-3 weeks She has been hospitalized on 50 occasions and has received altogether 29 pints of blood and 23 half-doses of fraction I/O She was always on iron therapy but was nevertheless often anaemic (Hb 9-10 g/100 ml) The menstrual flow became less excessive after infusion of plasma or fraction I/O or treatment with epsilon aminocaproic acid Of this drug the patient has received a total of more than 5000 g and this treatment of the patient has already been reported (19) During the last 12 months she has been successfully treated with gestagens Tooth extraction in 1965 was performed under cover of fraction I/O and epsilon aminocaproic acid without undue bleeding She never had joint bleeding and was otherwise healthy and well developed

Physical and laboratory investigation

Physical examination revealed no abnormalities Blood pressure 120/70 mm Hg ECG normal Chest X ray showed nothing remarkable ESR 7 mm/h Electrophoresis showed a normal pattern Her haemoglobin level was often low owing to bleeding or iron deficiency but otherwise her blood picture was normal The leucocyte count was about 5000/mm³ The patient belonged to blood group B CDe/cde M+ N+ S₁ P(+) K(-) Le(a-b+) Fy(a-) Coombs test was negative No irregular antibodies against red cells or leucocytes could be demonstrated

Widal's Wassermann's Waaler Rose's and Paul Bunnell's tests were negative as were antistreptolysin antistaphylolysin CRP cold agglutination tests and the gonococcal complement binding reaction Listeria agglutination occurred at a dilution of 1/64 and complement binding at 1/5

Coagulation studies

Coagulation analyses on more than 50 occasions in 1960-67 always gave consistent results (Table 1) The Duke bleeding time usually exceeded 30 min but was occasionally about 15 min on one side

Table I Coagulation analysis

	Patient	Range or standard error	No of investigations	Mother	Father	Maternal grand mother	Normal range
Bleeding time							
Duke min	> 30	10-30	30	3-4	-	-	1-4
Ivy min	> 30	> 30	4	8	-	15	6-15
Coagulation time							
glass min	13	7-19	9	12	6	12	8-14
plastic min	25	17-30	7	-	-	23	15-30
Prothrombin consumption	21	± 5.9	18	27	-	6	0-30
One stage prothrombin time sec	16	15.0-17.0	9	-	16.8	-	15-17
F VII + F IX + prothrombin (P&P) %	95	75-100	9	80	93	-	80-100
Recalcification time sec	144	120-180	9	-	183	-	100-160
F V	103	80-139	8	85	145	-	80-100
F VIII	87	± 5.4	35	71	120	131	60-160
F IX	80	62-105	7	92	-	95	60-160
F XI	100	-	-	-	-	-	60-160
Fibrinogen g/100 ml	0.33	0.20-0.39	7	-	-	-	0.26-0.34
Plasminogen activity	109	88-131	6	-	-	-	60-140
Urokinase inhibitors	90	77-104	4	-	-	-	60-140
Platelet factors 1 3 4	Normal	-	-	-	-	-	-
Platelet number per mm ³	185 000	± 6700	40	238 000	-	246 000	135 000-300 000
Platelet adhesiveness							
Hellem's whole blood	2	± 1.2	33	-	-	-	30 ± 4
Plasma + ADP 0.10 µg/ml	47	21-68	3	-	-	-	48 ± 15
0.05 µg/ml	3	10-15	3	-	-	-	26 ± 12
Salzman's method	9	3-17	4	-	-	-	0-60

The Ivy bleeding time always exceeded 30 min. The coagulation time and the various coagulation factors were normal. AHF (factor VIII) determined on 35 occasions was on the average 87% and factors IX, XI and XII were normal. No increased fibrinolytic activity was demonstrated in the blood samples. The patient had never shown signs of circulating anticoagulants.

The platelets were always normal in number as well as in appearance in the phase contrast microscope. They aggregated and adhered to glass in a normal way or possibly somewhat more strongly; this was apparent also when using Bredin's methods. Viscous metamorphosis after recalcification was normal. Platelet adhesiveness as tested with Hellem's whole blood method was 22 (mean of 33 determinations) i.e. bordered the lower limit of the normal range. After varying doses of ADP platelet adhesiveness according to Hellem's plasma ADP method was normal. The value found with Salzman's method was always decreased. Platelet aggregation in platelet rich citrate plasma was normal after addition of ADP in a final concentration of 1 µg/ml of ADP. The platelets also aggregated normally after ad-

dition of connective tissue suspension, dilute thrombin, trypsin or papain solutions. Clot retraction was normal. The activity of platelet factors 1, 3 and 4 was normal. Prothrombin consumption was mostly normal.

Familial investigation

Neither of the parents had had any bleeding symptoms and laboratory studies revealed nothing remarkable. The patient's maternal grandmother was also investigated and found to be healthy. There was no relative with a history of haemorrhagic diathesis. The patient had no sisters or brothers.

Therapeutical trials

The patient was repeatedly treated with whole blood and various kinds of plasma and plasma derivatives (Table II). Fraction I-0 shortened the Duke bleeding time and decreased clinical bleeding. The effect of one dose of fraction I-0 did not exceed 24 h. After the infusion the increase of factor VIII was that expected from the amount of factor VIII administered and there was no retarded increase. The administration of 400-1000 ml of fresh or stored plasma had a similar effect.

Table II *Effect of various infusions on the bleeding time of the patient*

Blood derivate	Dose (ml)	Total number of infusions	Effect		
			Good ^a	Moderate ^b	Bad ^c
Fraction I-O	100-200	11	8	3	0
Fresh II plasma	400-500	2	2	0	0
Stored II plasma	400-1000	9	8	0	1
Stored blood	450-900	5	2	1	2
Red cell concentrate	450-900	3	0	1	2
II serum	400-500	3	0	0	3
Platelet suspension	4 donors	1	0	0	1
Fresh plasma mixed blood group	300-500	4	0	1	3
Stored plasma mixed blood group	400-1000	11	4	6	1
Fresh B plasma from von Willebrand patient	400	1	0	0	1
Infusion of further normal stored II plasma	200	1	0	1	0
Stored O plasma of low antibody titre	550	1	0	1	0
Fresh frozen II plasma from patient with thrombasthenia	700	1	0	1	0
Stored A plasma adsorbed with red cells of blood group B	900	1	0	0	1
Albumin	5 g	2	0	0	2

^a Bleeding time shorter than 15 min in both ears^b Bleeding time 15-30 min^c Bleeding time exceeding 30 min for both ears.

which however was better and more consistent when the plasma belonged to the same blood group as the patient. Fresh plasma did not have a stronger effect than stored plasma. Occasionally a urticarial reaction occurred after the plasma infusion and then the bleeding time was never shortened. Serum, red cell concentrates or a platelet suspension from 4 donors were without effect.

Fresh plasma 400 ml from two patients with von Willebrand's disease and belonging to the same blood group was infused without subsequent shortening of the bleeding time. As the dose might have been too small, a further 200 ml of normal stored plasma from healthy donors belonging to the same blood group were later infused after which the bleeding time for one ear fell to 17 min.

Epsilon aminocaproic acid in a dose of 6 g four times a day had a favourable effect on her menstrual bleedings but not on the bleeding time. Treatment with prednisone 5 mg three times a day for one month did not affect her bleeding time or bleeding tendency. During the last 12 months she has been treated with gestagens: Anovlar mite (norethisteron 3 mg, ethinyloestradiol 0.05 mg) and the menstrual flow is no longer so profuse but the prolonged bleeding time and low adhesiveness with Salzman's method are unchanged.

DISCUSSION

The patient described had a severe haemorrhagic diathesis characterized by menorrhagia, nose bleeding and severe bleeding after cuts and dental extractions. The Duke and Ivy bleeding times were markedly prolonged but the coagulation time, prothrombin consumption and all coagulation factors were normal. There was no increased fibrinolysis. Platelet adhesiveness was low according to Salzman's method and near the lower limit of the normal range when tested with Hellem's method but the number, appearance and other reactions of the platelets were normal or if anything increased. When a drop of platelet rich plasma was placed on a glass slide the platelets showed normal or increased adhesion and spontaneous aggregation. Aggregation with ADP and connective tissue suspension was also normal. Platelet factors were normal. Viscous metamorphosis and clot retraction were also normal. Although the disease was obviously congenital, examination of her parents revealed nothing remarkable and no relative was known to have exhibited any bleeding symptoms. The Duke bleeding time and the bleeding symptoms decreased on treatment with fraction I-O, fresh or stored plasma especially when the plasma originated from a donor of the same blood group.

The fact that platelet aggregation and clot retraction were normal excluded Glanzmann's severe thrombasthenia. The normal prothrombin consumption and normal factor 3 and the good response to plasma infusions argue against thrombopathy or other primary platelet disorders. A prolonged bleeding time in association with decreased factor IX or XI has been described (3 8 13 14 15 30 32 33 34) but these factors were normal in this patient. Recently Hurst et al (18) described a patient with prolonged bleeding time and an abnormal response to connective tissue suspension but the possibility of such conditions was ruled out in our patient.

The condition resembled the severe form of von Willebrand's disease but not in all respects. There was thus no dominant heredity factor VIII was normal and in contrast with what might be expected from our experiences with patients with von Willebrand's disease the condition responded also to stored plasma. In mild von Willebrand's disease a normal factor VIII is occasionally found but the prolongation of the bleeding time, the bleeding tendency and the decrease in factor VIII are proportional and in a patient like ours a very low level of factor VIII was expected. No retarded increase in factor VIII was observed in the infusion trials.

It may therefore be concluded that the patient had a special hitherto unrecognized congenital bleeding disorder resembling but not identical with von Willebrand's disease. We have provisionally called the condition *Morbus Rata* after the Christian name of the patient. The symptoms, laboratory findings and presence of the factor in fraction I-O suggest that the disorder might be an isolated lack of the von Willebrand factor and the results of a trial in which plasma from von Willebrand patients failed to shorten the bleeding time argue for this possibility. However the results of a single trial should not be overestimated. The earlier finding that infusion of plasma from haemophilic patients raised the level of factor VIII in patients with von Willebrand's disease (21) suggests that the von Willebrand factor occupies an earlier position in a sequence of reactions than factor VIII and is of importance for the normal level of this factor. As factor VIII was normal in this patient the von Willebrand factor should therefore be normal too but some factor specifically responsible for maintenance of a normal

bleeding time and activated by the von Willebrand factor might be missing. Other mechanisms are also possible but investigations to elucidate the question were hampered by lack of appropriate *in vitro* methods to test the von Willebrand factor. No infusion of plasma from the patient was given to patients with von Willebrand's disease.

An interesting observation was that plasma from a donor belonging to the same blood group was more effective than mixed plasma. This suggests that the factor might possibly be inactivated in immunological reactions. The possibility of a connection between complement and opsonizing factors and factors associated with platelet adhesion and aggregation in the early stages of haemostasis has been suggested by Better-Galland and Lüscher (1) but the experimental and clinical evidence available in support of such an idea is so far meagre.

Bleeding should be treated by infusion of whole blood fraction I-O or plasma from a donor of the same blood group as the patient. Epsilon aminocaproic acid depresses the local fibrinolysis normally present in many tissues and controls menorrhagia. The menstrual bleeding responds also to gestagens.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Medical Research Council and from the Medical Faculty University of Lund, Lund, Sweden.

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TURNOVER OF ^{125}I AND ^{131}I LABELLED HAPTOGLOBIN IN MAN

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Abstract Haptoglobin catabolism has been studied in 17 subjects with the aid of ^{125}I and ^{131}I labelled haptoglobin. The catabolism has been found to be in the order of 10-20% of the IV pool per day—a catabolic rate significantly lower than that reported by previous investigators.

Plasma haptoglobin concentration varies widely in disease. It may be zero in hemolytic disease and is increased sometimes considerably in conditions with inflammation or tissue breakdown such as infarctions, collagen disease and malignant neoplasms (11-13). Haptoglobin metabolism has been studied mainly in connection with increased red cell destruction, a condition in which plasma haptoglobin exerts its physiological effect of binding plasma hemoglobin, thus preventing it from being excreted by the kidneys (2, 3, 9, 12). The possible importance of the increased haptoglobin levels in the other conditions mentioned is however entirely unknown.

We have studied the turnover rate of iodinated haptoglobin in hospitalized patients without signs of increased hemolysis.

MATERIAL AND METHODS

All subjects were hospitalized for at least three weeks during the study. Those labelled "normals" were patients admitted for routine investigations. None had signs of anemia or increased erythrocyte breakdown nor of recent acute disease. The ESR was normal or only slightly elevated (Table I). Patients: all with elevated ESR, had the following diagnoses: rheumatoid arthritis, two recent myocardial infarctions, one gastric ulcer, one acute gall bladder disease, one carcinoma of the colon, one acute deep vein thrombosis, one. None had signs of increased hemolysis, hemoglobins—with one exception—were within normal limits.

To ensure complete urine collection indwelling catheters were used. All subjects were given Lugol's solu-

tion (10 drops 3 times daily) at least three days before the start of the study. The radiolabelled haptoglobin preparation was injected intravenously. Blood samples were drawn 10 min after the injection and later every day at approximately the same time of the day. Urine was collected in 4 h samples.

Haptoglobin was fractionated from normal human plasma by the method of Stenbuck and Quenon (14). Further separation to obtain single polymers was done on Sephadex G-200 (3). Iodination was performed essentially according to the iodomonochloride technique of McFarlane (7). After iodination sterile filtration was performed through glass filters and tests were made to ensure that the preparations were sterile and pyrogen free. The hemoglobin binding capacity was tested after these procedures and was found to be unaltered. As a measure of catabolism the U/P ratio was used, i.e. the fraction of the dose excreted in the urine (=U) during 4 hours divided by the mean value of the fraction of the dose in the plasma pool (=P) during the same time according to principles set down by Campbell et al. (1).

Haptoglobin concentration was determined by Jayle's method as described by Nyman (11).

Alpha₂-globulin concentration was determined from paper electrophoresis.

RESULTS

The first set of experiments (A) was performed with ^{125}I , the second and the third (B and C) with ^{131}I . The slowly falling value of the catabolic rate (Fig. 1) in the first two sets (A and B) seems to show that at least some denaturation had occurred. The last two experiments (C) however have given absolutely constant catabolic rates indicating that the haptoglobin preparation was undenatured. Table I summarizes the results. Fig. 2 shows the typical disappearance curve in one of the last two experiments.

The haptoglobin catabolism in the first series of experiments amounted to on an average 20% of the IV pool per 24 hours. In the last two

Table I Clinical data and results of haptoglobin catabolism studies in ten normal subjects and seven patients

Pat. no	Sex	Age	Diagnosis	Hb (g/100 ml)	ESR (mm/h)	Serum hapto globulin (mg/100 ml)	Catabolism		
							α_2 globulin (g/100 ml)	iv pool/ day (%)	(g/day)
(A) ^{131}I									
1	o	22	Normal	13.8	12	—	—	16.3	
2	o	52	Normal	14.7	5	—	—	11.0	
3	♂	57	Normal	13.8	11	—	—	12.8	
4	♀	36	Normal	10.3	8	—	—	17.3	
5	♀	74	St.p myocardial infarction	12.7	42	—	—	18.7	
6	♂	46	Gastric ulcer	11.7	56	—	—	24.9	
7	♀	49	Acute arthritis (rheumatoid?)	10.7	96	—	—	23.6	
							Mean	17.8	
							Range	11.0-24.9	
(B) ^{125}I									
8	♀	83	Normal	13.2	38	160	0.45	18.4	0.7
9	o	66	Normal	14.0	3	79	0.45	21.4	0.5
10	o	56	Normal	14.3	27	244	0.56	20.8	2.1
11	♀	46	Normal	15.8	6	178	0.56	21.1	1.1
12	♀	64	Rheumatoid arthritis	11.9	102	363	0.89	17.2	2.4
13	♂	59	Leg thrombosis	11.4	98	342	—	25.2	3.5
14	♀	57	Colon carcinoma	8.8	72	300	—	21.3	1.6
15	♂	53	Acute gall bladder disease	11.4	110	600	1.05	21.9	5.1
							Mean	20.9	
							Range	17.2-25.2	
(C) ^{125}I									
16	♂	80	Normal (st.p slight cerebral vascular accident)	13.2	19	104	0.57	8.5	0.4
17	♀	81	Normal" (st.p slight cerebral vascular accident)	12.3	30	124	0.70	13.5	0.4

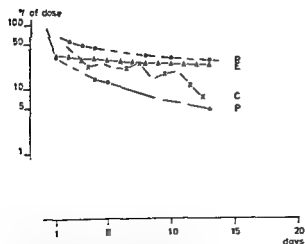


Fig 1 Haptoglobin catabolism (case 5) R retained dose E extravascular pool P plasma disappearance curve C catabolic rate (U/F) per cent per 24 hours (cf Methods)

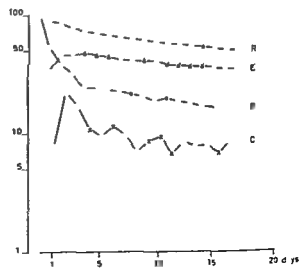


Fig 2 Haptoglobin metabolism in a normal man (case 16) Constant catabolic rate

experiments the rate of catabolism was found to be lower 9 and 14 % respectively

DISCUSSION

As stated in the introduction haptoglobin hemoglobin complex metabolism has been studied by several workers (2 4 6) However Freeman (3) found that haptoglobin catabolism was much less only one third of hemoglobin catabolism and concluded that it was possible that normally all haptoglobin was catabolised by a non-specific mechanism without combination with hemoglobin Garby and Noyes (5) in 1966 stated that the hemoglobin haptoglobin complex pathway was responsible for about half the total haptoglobin catabolism and in 1967 after further studies that 50-80% of the total catabolism of haptoglobin occurs by way of the hemoglobin pathway (10)

The purpose of this study was to analyze the haptoglobin catabolism in normals and in patients with elevated serum haptoglobin levels—without signs of increased red cell destruction As emphasized by Freeman (3) the plasma concentration of any protein is a balance between synthesis and catabolism The plasma concentration rises markedly in inflammatory conditions among others It is unknown whether this increase has any physiological meaning

Nyman (11) measured the disappearance of plasma haptoglobin after acute pneumonia in nine patients She calculated a half life for excess haptoglobin of 5.4 days (range 4.4-6.8) Morittu et al (8) reported a haptoglobin half life of 3.3 and 4 days in two subjects given ^{125}I labelled haptoglobin Freeman (3) in his study with ^{125}I labelled haptoglobin found an average catabolism of 36% of the i.v. pool per day (range 19-55) in normals and an increase up to 500% i.v. catabolism in patients with acute hemolysis In non hemolytic conditions he found no correlation between haptoglobin concentration and either hemoglobin concentration or rate of haptoglobin catabolism

In the majority of our experiments the average catabolism amounted to 20% of the i.v. pool per day (corresponding to a half life of 3.7 days) As stated above the slowly decreasing value of the catabolic rate (cf Fig 1) in experiments A and B might indicate that some denaturation had

taken place This type of curve however does not necessarily indicate denaturation but could be a sign of individual variations in the handling of "foreign" proteins In no experiments were there found any signs of strong denaturation with massive excretion during the first two days of the experiment

In the last two experiments (C) the catabolic rate was absolutely constant It was found to be lower than in the previous experiments amounting to only 8.5 and 13.5% of the i.v. pool per day

All the results would indicate a slower catabolism of haptoglobin than that found by Freeman (3) Against his average catabolism of 36% we found a catabolic rate between 10 and 20% Even admitting some denaturation in some of the experiments this would be a significant difference As Freeman (3) points out alteration of a protein during laboratory procedures will always induce an elevated never a decreased catabolism The low figures found here would therefore seem to be reliable

The differing results cannot be ascribed to differences in the patient material as the composition seems to be very similar in the two groups studied by Freeman (3) and ourselves It should be added that all subjects labelled normals were in steady state during the study

The lowest catabolic rate was found in two elderly subjects No studies however have been published to indicate that protein metabolism would decrease with age

In the patients with elevated ESR the haptoglobin catabolism was slightly more rapid (mean 22 range 17-25 per cent) than in the normal subjects (mean 16 range 9-21 per cent) This difference however was not significant

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Table I Clinical data and results of haptoglobin catabolism studies in ten

Pat no	Sex	Age	Diagnosis	Hb (g/100 ml)	ESR (mm/h)	Serum haptoglobin (mg/l)
(A) 131 _I						
1	♂	48	Normal	13.8	12	—
2	♂	52	Normal	14.7	5	—
3	♂	57	Normal	13.8	11	—
4	♀	36	Normal	10.3	8	—
5	♀	74	St p myocardial infarction	12.7	42	—
6	♂	42	Gastric ulcer	11.7	56	—
7	♀	49	Acute arthritis (rheumatoid?)	10.7	96	—
(B) 125 _I						
8	♀	83	Normal	15.2	38	160
9	♂	66	Normal	14.0	3	79
10	♂	56	Normal	14.5	27	244
11	♀	46	Normal	15.8	6	178
12	♀	64	Rheumatoid arthritis	11.9	102	363
13	♂	—	Leg thrombosis	11.4	98	342
14	♀	57	Colon carcinoma	8.8	72	300
15	♂	53	Acute gall bladder disease	11.4	110	600
(C) 125 _I						
16	♂	—	Normal (st p slight cerebral vascular accident)	13.2	19	104
17	♀	81	Normal (st p slight cerebral vascular accident)	12.3	30	124

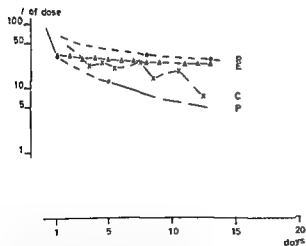


Fig 1 Haptoglobin catabolism (case 5) R retained dose E extravascular pool P plasma disappearance curve C catabolic rate (U/E) per cent per 24 hours (cf Methods)

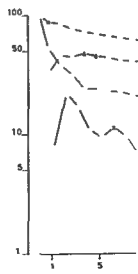


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In the majority of our experiments the average catabolism amounted to 20% of the iv pool per day (corresponding to a "half-life" of 3.7 days). As stated above the slowly decreasing value of the catabolic rate (cf Fig. 1) in experiments A and B might indicate that some denaturation had

taken place. This type of curve however does not necessarily indicate denaturation but could be a sign of individual variations in the handling of foreign proteins. In no experiments were there found any signs of strong denaturation with massive excretion during the first two days of the experiment.

In the last two experiments (C) the catabolic rate was absolutely constant. It was found to be lower than in the previous experiments amounting to only 8.5 and 13.5% of the iv pool per day.

All the results would indicate a slower catabolism of haptoglobin than that found by Freeman (3). Against his average catabolism of 36% we found a catabolic rate between 10 and 20%. Even admitting some denaturation in some of the experiments this would be a significant difference. As Freeman (3) points out alteration of a protein during laboratory procedures will always induce an elevated never a decreased catabolism. The low figures found here would therefore seem to be reliable.

The differing results cannot be ascribed to differences in the patient material as the composition seems to be very similar in the two groups studied by Freeman (3) and ourselves. It should be added that all subjects labelled normals were in steady state during the study.

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								iv pool/ day ()	(g day)
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6	♂	42	Gastric ulcer	11.7	56	—	—	24.9	
7	♀	49	Acute arthritis (rheumatoid?)	10.7	96	—	—	23.6	
								Mean	17.8
								Range	11.0-24.9
(B) ^{125}I									
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11	♀	46	Normal	15.8	6	178	0.56	21.1	1.1
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17	♀	81	Normal (st p slight cerebral vascular accident)	12.3	30	124	0.70	13.5	0.4

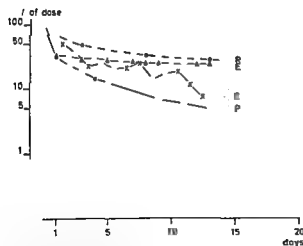


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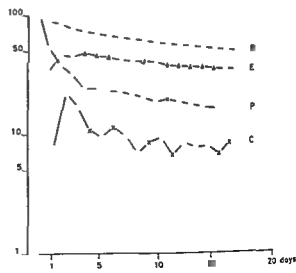


Fig 2 Haptoglobin metabolism in a normal man (case 16) Constant catabolic rate

STUDIES OF THE CLINICAL AND METABOLIC EFFECTS OF PHLEBOTOMY TREATMENT IN PORPHYRIA CUTANEA TARDA

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Göteborg Sweden*

Abstract The size of the iron stores and the effect of phlebotomy treatment has been evaluated in twelve patients with porphyria cutanea tarda. Clinical and biochemical remission was obtained in all patients. The mean treatment period was 4 1/2 months in patients who were phlebotomized frequently and were not given iron. In two patients who received iron supplements in conjunction with phlebotomy treatment remission was not obtained until the iron administration was stopped. In two other patients iron administration after induced remission was associated with relapses. No relapse was seen in the others. The effect of the treatment is probably due mainly to the reduction of the iron stores. Beside the disappearance of active skin symptoms and strongly diminished porphyrin excretion the treatment was associated with improved liver function tests.

Porphyria cutanea tarda symptomatica (PCT) is clinically characterized by skin fragility and photosensitivity. Blisters often appear on sun-exposed areas. These frequently ulcerate and lead to scar formation. Hypertrichosis and hyperpigmentation are common.

Chemically the disease is characterized by the excretion of large amounts of uroporphyrin (UP) and lesser amounts of coproporphyrin (CP) in the urine. The excretion of delta aminolevulinic acid (ALA) may be slightly increased but the excretion of porphobilinogen (PBG) is normal. The faecal excretion of CP and protoporphyrin (PP) is usually slightly increased.

Laboratory signs of disturbed liver function (35) are usually present and decreased glucose tolerance (32) is common. Microscopic examination of liver biopsy specimens often reveals steatosis, fibrosis or cirrhosis (35).

The disease generally manifests itself in middle or late life, its peak incidence being in the fifth decade. Males are much more commonly affected than females. PCT is usually considered to be

acquired and according to the literature most patients are alcoholics. In some cases however there is a family history (9, 10). Watson (37) has stressed the importance of constitutional (here dietary?) factors that appear to be necessary for the manifestation of the disease.

Earlier treatment of the disease has been entirely symptomatic. The importance of forbidding alcohol consumption is usually stressed. Treatment with chelating agents (24), chloroquine (7), cholestyramine (31) and adenosine monophosphate (8) has been reported to be associated with improvement of cutaneous symptoms in some cases.

Ippen was the first to report beneficial effects of phlebotomy treatment in PCT (14, 16). Similar results have been reported by others (6, 17, 29, 30). In most series however it is difficult to evaluate the effectiveness of phlebotomy treatment as other factors including stopping drinking and hospitalization may have resulted in improvement of the disease. Furthermore the way in which phlebotomy treatment affects the disease and particularly whether the effect of the treatment is due to reduction of iron stores in the liver or to an increase in hemoglobin production is not known. Increase of histochemically demonstrable iron in liver cells has been reported (3, 34). The estimation of histochemically demonstrable iron in the liver is however an unreliable measure of total liver iron concentration (39).

The main aim of the present study was to evaluate the effectiveness of phlebotomy treatment to quantitate the iron stores in PCT and to determine whether the effect of phlebotomy treatment is related to reduction of the iron stores. Furthermore to study the biochemical changes

Table I Duration of clinically manifest disease at the start of treatment alcohol consumption and porphyrin excretion before and after treatment

Pat	Sex	Age (y)	Duration (y)	Alcohol consumption (l abs alcohol/month)		Porphyrin excretion before and after treatment							
				Before treatment	During treatment	Urine ($\mu\text{g}/24 \text{ h}$)				Faeces ($\mu\text{g/g}$ dry wt)			
						UP (N v < 100)		CP (N v < 150)		CP (N v < 20)		PP (N v < 40)	
						Before	After	Before	After	Before	After	Before	After
E S	♂	47	4½	0.9	0.2	4 280	340	160	20	158	8	127	25
H P	♂	42	2½	1.4	0.3	2 800	70	360		111	2	7	3
S B	♂	53	½	0.8	0.8	2 800	370	110	150	82	5	111	21
G S	♂	50	14	1.6	1.6	3 180	240	510	50	128	22	129	25
G A	♂	50	2	4.5	2.5	3 420	310		40				
T S	♂	49	1	0.2	0.0	2 080	220	230	40	115		92	
B C	♂	64	1	0.6	0.6	10 970	530	530	20	134		186	
J K.	♂	50	1½	0.0	0.0	6 330	800	340	50				
H G	♂	57	½	0.6	0.6	2 420	730	250	50	8		15	
A G	♂	40	2½	0.2	0.0	3 510	400	460	160	80	13	73	7
J E	♀	5	0.0	0.0	0.0	4 720	940	380	40	352	2	311	6
M I	♀	23	½	1.5	0.2	3 620	710	130	30	78		83	
Mean		49	3			4 180	470	315	59	116		111	
Range						10 970-2 080	940-70	530-110	160-20	352-8		311-7	

occurring during phlebotomy treatment with respect to porphyrin excretion liver function and glucose tolerance

Twelve patients with typical PCT of whom six did not alter their alcohol consumption were studied. The hospitalization was short and no other form of treatment for their disease was given.

MATERIAL

The series includes nine men and three women (Table I) all with typical clinical and biochemical signs of PCT. Their ages ranged from 23 to 64 years (mean 49 years). Skin fragility and ulcers were consistent symptoms. Most patients had vesicles. Gross sclerodermatoid changes were seen only in one patient (J E). Two patients (B C, J K.) had overt diabetes mellitus requiring treatment.

The patients were questioned about alcohol consumption several times. Except for one (B C) reliable information probably has been obtained. The amount of alcohol consumed (Table I) has been transformed to absolute alcohol. One patient (J E) was a teetotaler and one patient (J K.) consumed insignificant amounts. Moderate quantities were consumed by two patients (A, G, T, S). The rest of the patients consumed large quantities but none was alcoholic from the social point of view. Only one consumed significant quantities of wine (H P).

The youngest of the patients (M I) was started on norethandrone (2 mg daily) and mestranol (0.1 mg daily) as contraceptive agents in November 1965. Skin symptoms

appeared in July 1966. The hormone treatment was stopped in September 1966 and the phlebotomy treatment was started five months later. It is possible that the treatment with anticonceptive agents may have elicited the disease. Appearance of porphyric disease during sex hormone treatment has been reported (41).

The patient (J E) who never had consumed alcohol had epileptic attacks and had been treated with hydantoin derivatives for several years. One patient (B C) was being treated with chlorpropamide and one (J K.) with tolbutamide. One patient (H P) probably had infectious hepatitis 15 years ago. No patient had been treated with antileptic agents.

Two patients (G, S, G, A) were identical twins and have been reported on earlier by Hæger Aronsen (9). Otherwise there was no family history suggestive of porphyria.

METHODS

Urinary excretion of UP and CP was determined by the technique of Askevold (1). To ensure conversion of CP precursor to CP, iodine wash according to Watson et al (36) was used. The urine was collected in bottles supplied with 5 g of sodium carbonate and stored cold or frozen. Quantitative determinations of CP and PP in faeces were made according to the method of Holt et al (11). The calculations were made by the formula given by Rungtun and Svemsson (76). The extinction constants and corrections recommended by Wuth (40) were used. Quantitative urinary ALA and PBG determinations were performed according to Mauzerall and Granick (73).

Bilirubin and alkaline phosphatases in serum were determined according to a modification by Roos (77).

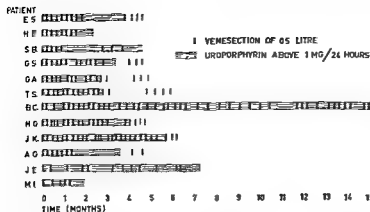


Fig 1 Phlebotomy program and time required until the UP excretion in urine had fallen to one mg per day. C was given iron sulphate orally (300 mg/day) for 6 months. H G was given iron dextran intravenously during a period of 10 weeks and the amount given was equal to the calculated iron content of the removed blood (2700 mg).

thymol turbidity according to MacLagan (1). Serum glutamic oxaloacetic transaminase (SGOT) was determined in a Technicon autoanalyzer according to a modification of the method of Babson et al. (2). The bromsulphalein dye retention test (BSPT) was carried out by the technique of Rosenthal and White (3) but 5 mg of the dye per kg body weight was given and the retention was determined after 45 min. Intravenous glucose tolerance test was performed according to Ikko and Luft (13).

Hemoglobin was determined photometrically as cyanmethemoglobin. Serum iron (Fe/s) was determined by the method of Laurell (38). The total iron binding capacity of serum (TIBC) was determined according to Peters et al. (3). The desferrioxamine test was performed according to Lundvall and Weinfeld (4). Ten mg of desferrioxamine per kg body weight was administered intramuscularly and the desferrioxamine-induced iron excretion (Fe/urine) was determined in urine collected for 24 hours after the desferrioxamine injection.

Liver biopsy was performed in ten patients. In two it had been performed some years before this study (G S 1960, H P 1961). In the last patient (H P) the biopsy specimen was taken surgically at operation for duodenal ulcer (subtotal gastrectomy). Otherwise the biopsy specimens were obtained by the percutaneous aspiration technique. A small piece of the specimen was fixed in ten per cent neutral formaldehyde embedded in paraffin and made into 3 μ sections for histological examination. Hemosiderin was stained with potassium ferrocyanide according to Hutchison (12) and graded on an arbitrary scale of 0-4+ according to the principles previously described for bone marrow sections (19). The major part of the biopsy was freeze-dried and after determination of dry weight analysed for its total non-haem iron content essentially according to the method previously described (38). To render analysis of the small material obtained by percutaneous liver biopsy (mean dry weight about 100 mg) possible the extracts were diluted to 10 ml. Proportionally less of the reagents were used.

The phlebotomy treatment (Fig. 1)

Blood was removed by serial phlebotomies of 500 ml each. At each phlebotomy hemoglobin was determined. Serum iron and TIBC were determined at each or every other phlebotomy. The phlebotomies were repeated at

weekly intervals until the hemoglobin concentration one week after the last phlebotomy had fallen to 110 g per 100 ml or below this level. When this range was reached the hemoglobin concentration and the serum iron were determined weekly for three or four weeks. If during this period the hemoglobin level remained unchanged and the serum iron concentration was persistently low it was concluded that the iron stores were depleted. If the hemoglobin concentration showed a significant rise within three to four weeks the phlebotomies were repeated until signs of depleted iron stores according to the above criteria were achieved. In some patients the performance of the complete phlebotomy program was not possible without temporary interruption and in one (J E) the phlebotomies could be made only once or twice monthly. The number and frequency of phlebotomies for each patient are shown in Fig. 1. Two patients were given iron in conjunction with the phlebotomy treatment. Patient B C was given iron sulphate orally for 6 months (300 mg/day). Patient H G was given iron dextran intravenously after each phlebotomy during a period of ten weeks. The amount of iron administered to the latter was equal to the calculated iron content of the removed blood and the total amount of iron dextran given was 2700 mg. He was phlebotomized more frequently than the other patients (5-6 times monthly). Two patients (S B and A G) were given iron after a completed phlebotomy program.

The desferrioxamine test was performed initially in all patients and then at regular intervals. Porphyrin excretion in urine was determined before treatment and followed every week or every other week during treatment. Porphyrin excretion in faeces was determined before and after the accomplished course of phlebotomy treatment.

The patients were informed as to the probable connection between the disease and alcohol consumption. They were not forbidden to consume alcohol but told to report their alcohol consumption. Six patients did not alter their alcohol consumption, two (T S A G) stopped consuming alcohol entirely and the rest of the patients reduced their alcohol consumption more or less (Table I).

The chlorpropamide and tolbutamide treatment in the two patients with diabetes mellitus and the hydantoin treatment in the patient with epilepsy were continued during the phlebotomy treatment.

No patient received any other form of treatment for

Table II Liver histopathology before treatment and liver function tests before and after phlebotomy treatment

Pat.	Liver histology	Bilirubin/s (mg/100 ml)		GOT/s (units)		BSPR (retention)		Glucose tolerance (k value)	
		N v < 1.1 Before	After	N v < 40 Before	After	N v < 5 Before	After	N v > 1.0 Before	After
E. S.	Steatosis	0.5	0.4	48	29	16	7	0.75	1.31
H. P.	Fibrosis periportal round cell infiltration	1.1	0.7	116	88	34	11	1.08	
S. B.	Steatosis	0.5	0.6	44	24	6	3	0.70	0.94
G. S.	Steatosis	1.4	0.7	60	39	19	11	0.83	1.25
A. A.		1.4	1.2	85	45	25	10	1.07	
T. S.	Steatosis	1.0	0.4	44	40	10	6	1.31	
B. C.	Steatosis fibrosis periportal round cell infiltration	0.7	0.2	51	30	9	5	Overt diabetes	
H. G.	Steatosis	0.6	0.2	44	21	8	6	1.61	
J. A.	Steatosis fibrosis periportal round cell infiltr	0.7	0.4	56	19	10	6	Overt diabetes	
A. G.		1.1	0.4	72	24	3	1	0.64	0.85
J. E.	Normal	0.3	0.4	40	43	10	9	0.82	1.17
M. I.	Normal	0.5	0.3	65	21	14	3	1.51	
Mean		0.8	0.5	60	35	14	7		
Mean difference		0.3		25		7			
S.E. of mean difference		0.1		4		2			

their porphyric disease. One patient has been hospitalized for many years because of epilepsy (J. E.) but no other patient was hospitalized longer than was necessary for the examinations (1-2 weeks). The treatment was generally started 1-2 weeks after the discharge from the hospital.

RESULTS

Laboratory Findings Before Phlebotomy

Porphyria analyses (Table I)

All patients excreted large amounts of UP in the urine (2080-10 970 μg per 24 hours with a mean value of 4180) and the excretion of CP in the urine was usually moderately increased (110-530 μg per 24 hours mean 315). The faecal excretion of CP and PP was also increased in nearly all patients and ranged from 8 to 352 (mean 59) and from 7 to 311 (mean 111) μg per g dry weight respectively. The urinary excretion of PBG and ALA was normal in all.

Liver histopathology and liver function tests (Table II)

Two of the ten patients in whom liver biopsy was performed had normal liver histology. The other

eight showed varying degrees of steatosis and/or fibrosis. None had overt cirrhosis. Inflammatory changes were seen in three patients.

Serum bilirubin was borderline or above normal in five patients. The SGOT ranged between 37 and 116 units with a mean of 60 units. The BSPR was abnormal in all but one. The alkaline phosphatases and the prothrombin time were normal in all. Paper electrophoresis showed normal serum albumin values in all but one (J. E.) who also had an increased gamma globulin fraction and increased thymol turbidity.

In the patients without overt diabetes mellitus the intravenous glucose tolerance test showed decreased tolerance in five and normal in five.

Iron stores (Table III)

Signs of disturbed iron metabolism were seen in nearly all patients. The serum iron level was high in most subjects and the mean value was significantly higher than that of male controls. The TIBC level was within the range of normal except in one (J. E.). The mean desferrioxamine induced urinary iron excretion of the porphyric group was significantly higher than that of a control group comprising 25 healthy male sub-

Table III Iron studies in serum and liver

Pat	Sex	Hb (g/100 ml)	Fe/s (μ g/100 ml)	TIBC (μ g/100 ml)	TIBC saturation (%)	Fe/urine (mg/24 h)	Storage iron in the liver		
							Chemical determination of non hemin iron (mg/100 g dry wt)	Histochemical estimation of stainable iron (grade 0-4+)	In parenchymal cells
E S	♂	14.4	168	373	53	1.30	232	+++	+++
H P	♂	15.0	210	341	62	1.42	206	+++	+++
S B	♂	14.6	133	373	36	0.73	80	++	++
G S	♂	15.8	189	377	36	2.21		+++	+++
G Å	♂	16.7	156	468	58	2.28			
T S	♂	14.5	259	326	79	1.30	163	+++	++
B C	♂	15.4	207	269	76	2.60	128	+++	++
J K	♂	14.2	300	331	91	4.33	598	+++	+++
H G	♂	14.8	247	357	69	0.64	62	++	+
A O	♀	13.6	206	284	78	1.89			
J E	♀	13.4	114	204	56	0.71	245	+++	+++
M I	♀	12.8	206	315	65	0.67		++	++
Mean \pm s.e.			200 \pm 15	314 \pm 14	63 \pm 3	1.67 \pm 0.3	94 \pm 60		
Normal men									
Mean \pm s.e. (number)			133 \pm 8 (25)	331 \pm 9 (25)	41 \pm 3 (25)	0.76 \pm 0.04 (25)	97 \pm 9 (18)		
Range			72-275	264-417	25-79	0.47-1.27	45-192		

jects. The urinary iron excretion was above the range of the control group in seven of the men and in one of the women.

Non hemin iron in liver biopsy specimens was determined in eight patients. Values above the normal range were found in four of them. The mean value was 214 mg per 100 g dry weight. The range (62-598 mg) was however wide. The mean value in a control group comprising 18 males was 97 mg with a range of 44-192 mg per 100 g dry weight.

Histochemically demonstrable iron was present in both parenchymal cells and Kupffer cells in all porphyric subjects studied. In parenchymal liver cells three had stainable iron of grade 2+ and six of grade 3+. Only one (J K) had stainable iron of the highest grade (4+). In Kupffer cells one had stainable iron of grade 1+ four 2+ and five grade 3+. In a control series comprising 20 normal men stainable iron in parenchymal liver cells was found in 15. Grade 1+ was found in eight, grade 2+ in five and grade 3+ in two subjects. In the control series stainable iron in Kupffer cells was demonstrated in only three subjects (two had grade 1+ and one grade 2+).

Effects of Phlebotomy Treatment

Two to 14 (mean 7) litres of blood were removed from patients who did not obtain iron supplements during phlebotomy treatment (Table IV). The mean period of treatment for the same patients was 4.3 months.

Clinical signs and porphyrin excretion

Phlebotomy treatment was associated with loss of active porphyric skin symptoms in all treated patients. Thus skin fragility, ulcers and blisters consistently disappeared during the treatment.

A marked decrease in porphyrin excretion accompanied the phlebotomy treatment in all patients (Table I, Figs 1-3). The UP excretion in urine diminished to about 1/10 of the original amount. In one patient (H P) the UP excretion was normalized. In most patients the CP excretion in urine diminished and became normal. The faecal excretion of porphyrin diminished in all patients examined.

The clinical remission roughly coincided with the time when the urinary porphyrin excretion fell to about one mg per day (Fig 2). With two exceptions (J E, B C) this occurred 2-6 months

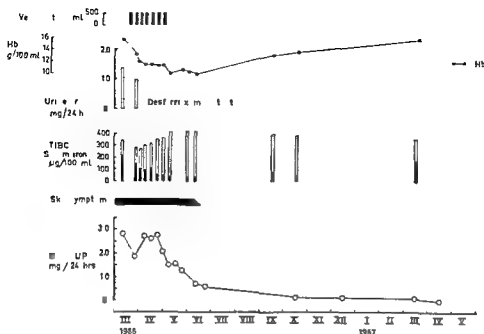


Fig. 2. Biochemical changes during phlebotomy treatment in a 47 year old man (H P) with porphyria cutanea tarda.

after the commencement of phlebotomy treatment. Longer periods of treatment were required in the patient (J E) who could be phlebotomized only once or twice monthly and in the patient (B C) who was given iron orally in conjunction with phlebotomy treatment. The last patient had the longest treatment (15 1/2 months) and he did not improve until the iron administration was

withdrawn and the iron stores were depleted. In the patient (H G) who was given iron dextran intravenously the porphyrin excretion diminished temporarily during intensive phlebotomy treatment. Later however it increased continually in spite of the phlebotomies. The iron administration was then stopped. After one more month of phlebotomy treatment the UP excretion fell below 1 mg per day and he became free of symptoms.

Most patients tolerated the treatment well but some felt tired and had symptoms from the induced iron deficiency anaemia.

During follow up periods of 6 to 18 (mean 10) months no relapse was seen in any of the patients who did not obtain iron after induced remission. In one patient (A G) oral iron administration was started about eight months after induced remission (Fig. 3). She was given 400 mg of iron sulphate daily for 30 days and then 300 mg daily for 15 days. The urinary UP excretion showed increasing values after 3 1/2 months and after two months more the UP excretion increased to about 1.5 mg per day and subsequently rose to about five mg per day. She has now again been treated with phlebotomies and the UP excretion has diminished (Fig. 3). Another patient (S B) was given 1100 mg iron dextran intravenously

Table IV. Amount of removed blood, treatment period and time required until the UP excretion in urine had fallen to one mg per day.

Patient	Blood removed (l)	Treatment period (mo)	Period with excretion in urine above 1 mg/day (mo)
E S	6.9	4 1/2	4
H P	4.2	1 1/2	2 1/2
S B	4.2	3 1/2	4 1/2
G S	8.0	4 1/2	3 1/2
G A	8.2	5	3
T S	10.2	6	3
B C	27.6	15 1/2	16
H G	11.0	5	4
J K	14.3	6 1/2	6
A G	4.7	4 1/2	3 1/2
J E	6.9	8 1/2	8 1/2
M I	2.1	1 1/2	2

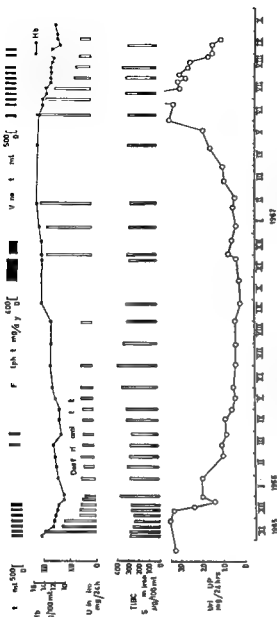


Fig. 3 Biochemical changes during phlebotomy treatment and after oral iron administration in a 40 year old woman (A, O) with porphyria cutanea tarda. Note the effect of oral iron administration on the desferrioxamine induced urinary iron excretion and the excretion of uroporphyrin.

eight to nine months after induced remission. Increased UP excretion was noted after four months with a subsequent rise to about seven mg per day.

Liver function tests and glucose tolerance (Table II)

The liver function tests were improved after treatment. The serum bilirubin level was significantly lower and all had values below 0.3 mg per 100 ml after treatment. Thymol turbidity, serum electrophoresis, prothrombin time and alkaline phosphatases were essentially unchanged. The bromsulphalein retention diminished in all and became normal in two patients. The SGOT diminished or became normal in nearly all patients. It persisted on a high level after treatment in only one patient (H.P.). He probably had infectious hepatitis 15 years earlier.

In one of the diabetic patients the tolbutamide dosage could be reduced from 0.5 g twice a day to 0.5 g once a day without increase in the blood sugar level. In the other patient with overt diabetes the chlorpropamide dosage was unchanged during treatment. In the five others with decreased glucose tolerance this was improved in all after phlebotomy treatment.

DISCUSSION

Signs of disturbed iron metabolism in PCT have been reported. High serum iron values are common findings (18). Histochemically visible iron in the liver seems to be present in most cases (3-34). Stainable iron in the liver was however a normal finding in a male control series. In the individual case the histochemical estimation of stainable iron is an unreliable measure of the iron content of the liver (39). However, the porphyric group in this study had more stainable iron than a control series. They differed strikingly from the control series with regard to stainable iron in Kupffer cells. Furthermore, four out of eight porphyric patients examined had a non-hemin iron content in the liver which was above the normal range. None had a liver iron concentration of the magnitude encountered in fully developed idiopathic hemochromatosis. Increased desferrioxamine induced iron excretion was found in eight out of twelve patients.

Increased iron stores in PCT have been thought

to be due to increased intake of iron in alcoholic beverages (18). A number of wines contain significant quantities of iron. Furthermore some recent experiments show that alcohol increases the absorption of ferric iron (5). In this series however only one patient was a wine drinker. The woman (J E) who was a teetotaler had a high liver iron concentration. Moreover in a series of advanced chronic drinkers of distilled spirits we rather found decreased iron stores (21). Hence we consider that the increased iron stores in PCT in this series was unrelated to alcohol consumption. Increased absorption of food iron or faulty release of iron is more probable.

The phlebotomy treatment was associated with loss of active porphyrin skin symptoms in all patients and the porphyrin excretion in urine decreased to about one tenth of the original amount. Spontaneous remissions occur and in alcoholics who stop drinking the porphyrin excretion may become normal (35). It is possible that some of the patients in this series improved because of diminished alcohol consumption. Most of our patients however continued to consume alcohol and in six of them alcohol consumption was unchanged. The course was the same and all improved. Not only did disappearance of skin symptoms and decreased porphyrin excretion accompany the phlebotomy treatment but the liver function tests also improved in all. The improved tolerance was perhaps also due to improved liver function. The favourable effect on liver function tests was also noted in the patients with unchanged alcohol consumption.

The way in which phlebotomy treatment affects the porphyrin metabolism in PCT is not clear. Ippen (15) who introduced the phlebotomy treatment in this disease has put forward the theory that the pathological porphyrins and their precursors take part in the compensatory formation of new hemoglobin. He does not believe in a metabolic block in hemoglobin synthesis since anaemia is not a feature of the disease. However the existence of an enzymatic block for hemoglobin synthesis located solely in hepatic cells might be considered. Such a hepatic block might explain the accumulation of porphyrins and would not necessarily imply defective hemoglobin production in the bone marrow.

The effect of the phlebotomy treatment may be related to the reduction of iron stores. In

favour of this view is the fact that the UP excretion remained at a high level in the two patients who were given iron in conjunction with phlebotomy treatment. Furthermore the only patients who had relapses were those who were given iron after induced remissions. The last argument must however be evaluated with caution as only two of the other patients had observation periods of the same length as the two patients given iron after remission. Signs of increased iron stores were present in most of our patients. However some who had normal liver iron concentrations also improved and it is probable that even normal amounts of iron in the liver cells may have a noxious influence on the porphyrin metabolism in porphyrin individuals. As PCT is uncommon in iron storage disease it is however probable that disturbed iron metabolism is not the primary cause of the disease.

The improved liver function tests after treatment may imply that the accumulation of porphyrins and iron in the liver cells may have a noxious effect on liver cell function. Therefore it seems indicated to phlebotomize also patients with latent PCT. The frequent finding of hepatic carcinoma in the autopsy series of Braun and Berman (4) favours this view.

ACKNOWLEDGEMENT

This work was supported by the Swedish Medical Research Council (Project No. 25x 593-07).

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COMPARATIVE STUDIES ON INTRAMUSCULAR AND ORAL EFFECTIVE DOSES OF SOME ANTICHOLINERGIC DRUGS

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Abstract. Sixteen healthy volunteers received propantheline, methylscopolamine or butylscopolamine in oral as well as in administration. After propantheline and methylscopolamine salivary secretion was found to be more influenced than the heart rate and the near point of accommodation. The relations between in and oral doses producing comparable total effects on salivation were 1:10 for propantheline and 1:100 for methylscopolamine. After oral butylscopolamine in doses up to 480 mg no anticholinergic effects were observed. In contrast to the other compounds in butylscopolamine produced only transient effects and influenced mainly the near point of accommodation. It is concluded that presumably less than 10 per cent of methylscopolamine and probably only very little of butylscopolamine is absorbed in the gastrointestinal tract.

The ratio between parenteral and oral effective doses of drugs reflects some of their pharmacological properties, among which the gastrointestinal absorption may be the most important. Although such a determination is relatively simple to perform, published observations of this kind are few.

In this investigation effective intramuscular and oral doses of propantheline, methyl- and butylscopolamine were studied in man on the salivary secretion, the heart rate and the accommodation of the eye. The responses to the two different administrations of each drug were compared quantitatively in the same subjects.

MATERIAL AND METHODS

Fifty-one experiments were performed using 16 healthy volunteers aged between 20 and 35, comprising nine females and seven males. The salivary secretion, the heart rate and the near point of accommodation were studied in the following manner. The salivary secretion was stimulated by having the subject chew a tablet of

ascorbic acid (250 mg) during 30 sec. The saliva obtained including that produced during the following 30 sec was spat out and the total volume was measured as previously described (11). In this paper the term salivary secretion refers only to saliva obtained in this manner. The heart rate was taken as being that of the frequency of the radial pulse. The near point of accommodation was determined as the shortest distance at which the subject was able to read very fine print with one eye. In this test each subject consistently used the same eye.

The drugs were administered after the subjects had rested in a sitting position for 40 min. During this time three determinations of the three parameters were made at intervals of 15 min. At the beginning of the investigation it was noted that the first of the three determinations of salivary secretion constantly showed a lower value than the following two and so this value was excluded. At the end of the investigation when the intramuscular route was used with the same subjects all three control values were similar.

Oral administration was in the form of commercially available tablets. The parameters were continuously determined at hourly intervals during the following eight hours. Placebo tablets containing lactose were also given. The experiments were carried out with a double blind technique.

The injections were given in the thigh. The parameters were determined at intervals of 15 min during the first hour at intervals of 30 min during the second hour and at hourly intervals subsequently up to and including the eighth hour after the administration of the drug.

The subjects were either fasting or had had a light breakfast about two hours before the experiment. They usually had a light lunch three or four hours after the drugs had been given. The drugs studied, the doses used, the number of experiments and the compound each subject received are shown in Table 1. One subject received atropine. The effects of oral administration of propantheline and methylscopolamine were studied in 11 subjects. The responses to intramuscular administration were studied on four of these subjects.

Quantitative comparisons between the effects of intramuscular and oral administration of the drugs were carried out as follows. The mean of each parameter for the predrug estimations was drawn as a continuous baseline

Table I *Participating subjects on each drug route of administration and dose*

Drug	Oral dose (mg)	Subjects	Intramuscular dose (mg)	Subjects
Placebo (lactose)		RS HB IW PH EK		
Atropine sulphate (Atropin ACO)	2	IJ	0.33	IJ
	4	II	1.0	IJ
			3.0	IJ
Propantheline bromide (Pro Banthine Searle)	30	CH BK EK PH	3	CH BK
	60	CH BK EK PH	10	CH BK
	120	CH BK EK PH		
Methylscopolamine nitrate (Skopyl Pharmacia)	4	TM III RT BMC EW	0.16	CO JM
	8	CO JM RT BMC	0.50	CO JM
	16	CO JM RT BMC		
Butylscopolamine bromide (Buscopan Boehringer/Ingelheim)	240	IW KÖ	10	IW KÖ
	480	IW KÖ	30	IW KÖ

on a diagram. The hourly post administration values which formed an effect curve were entered on this diagram. The area between the baseline and the effect curve was measured planimetrically for each subject, parameter and dose. These areas were then compared.

Student's *t* test was used when a statistical analysis of the material was performed.

RESULTS

The placebo group had a constant response to stimulation of salivary secretion during the eight hours of the experiment (Fig. 1). The heart rate showed no noteworthy variations with the exception of a slight increase at the determination that followed lunch (the fourth hour). The near point

of accommodation was increased in some of the subjects at the last two determinations.

Atropine 2 mg orally caused a moderate inhibition of the salivary secretion within one hour. A 4 mg oral dose produced a greater inhibition at this time. However, during the period of eight hours there was no significant difference between the total effects of the two doses (Fig. 2a). Intramuscular administration of 3 mg of atropine caused a corresponding degree of inhibition of salivary secretion during the eight hour period (Fig. 2b). During the same period of observation the effect on heart rate of 4 mg of atropine orally was approximately equal to that of 1 mg intra-

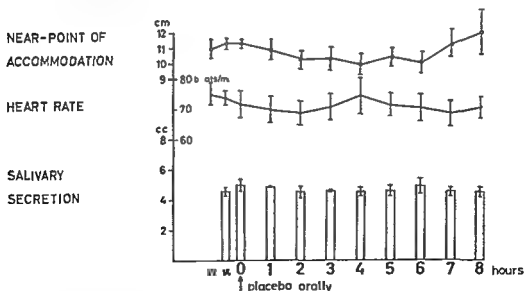


Fig. 1 The influence of oral placebo on three cholinergic functions in five subjects (means \pm S.E.).

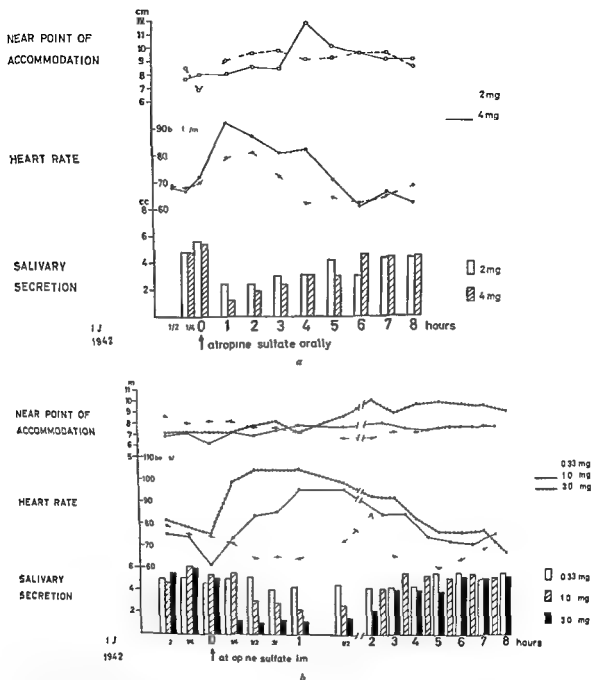


Fig 2 (a) Anticholinergic effects of atropine 2 and 4 mg orally in subject I J (b) Anticholinergic effects of atropine 0.33 1.0 and 3.0 mg im in subject I J

muscularly. The near point of accommodation tended to increase after the largest doses of atropine (Fig 2 a and b).

Propantheline 30 mg orally caused a slight decrease in the salivary secretion. Sixty mg and

120 mg resulted in a substantial inhibition of the secretion ($p < 0.01$) and in the higher dose in tachycardia as well ($p < 0.05$) (Fig 3). No effect on the near point of accommodation was observed in two subjects while in the remaining two the

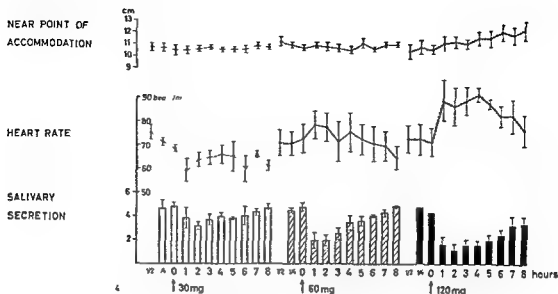


Fig 3 Anticholinergic effects of propantheline 30 60 and 120 mg orally in four subjects (means \pm s.e.)

distance increased moderately after the highest dose

Thirty mg of propantheline orally depressed salivation during eight hours approximately as much as 3 mg intramuscularly in the same subjects. The effect of 60 mg orally corresponded rather well to that of 10 mg intramuscularly. The results from one of the two subjects are shown in Fig 4a and b. The other subject showed similar results. Propantheline 120 mg orally increased the heart rate in both subjects to the same degree as 10 mg intramuscularly.

Methylscopolamine 16 mg orally caused an inhibition of salivation ($p < 0.05$) and a tachycardia (Fig 5). It was found that the effect on salivation obtained with this dose corresponded quantitatively to that of 0.16 mg administered intramuscularly (Fig 6a and b). In one subject the increase in the heart rate following 16 mg of methylscopolamine orally was somewhat less than that of 0.50 mg intramuscularly (Fig 6a and b). In the other subject 16 mg orally increased the heart rate half as much as 0.50 mg intramuscularly. At the doses used neither oral nor parenteral administration had any effect on the near point of accommodation.

Butylscopolamine in the doses of 240 and 480 mg orally and 10 mg intramuscularly did not influence salivation, heart rate or the near point of accommodation. The results from one of the

subjects are shown in Fig 7a and b. Thirty mg intramuscularly caused in this subject a slight inhibition of salivary secretion, an increase in heart rate and a very pronounced increase in the near point of accommodation. In the other subject the effects were quantitatively less. In both subjects the effects were of very short duration.

Symptoms. None of the subjects in the placebo group experienced any symptoms. The others felt dryness of the mouth simultaneously with demonstrable inhibition of salivation. In two subjects this feeling was reported without measurable salivary inhibition. When the decrease of the salivary secretion was pronounced the subjects also complained of roughness or slight pain in the throat. One subject reported tired eyelids after 16 mg of methylscopolamine orally while another complained of headache after 0.5 mg intramuscularly. One subject became drowsy after 10 mg of propantheline, another after 10 mg of butylscopolamine intramuscularly.

DISCUSSION

As the results are based on repeated measurements of three parameters during more than eight hours it was considered important to evaluate the possible appearance of other than drug-induced fluctuations in the parameters. In the placebo studies no such significant variations were ob-

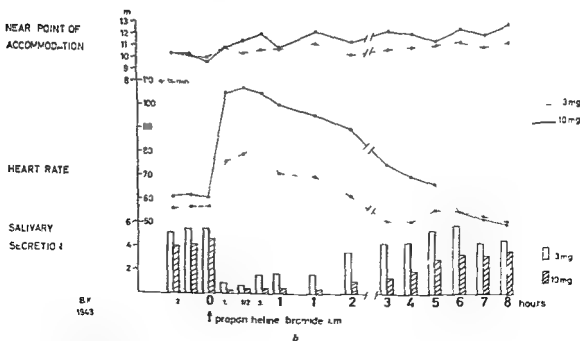
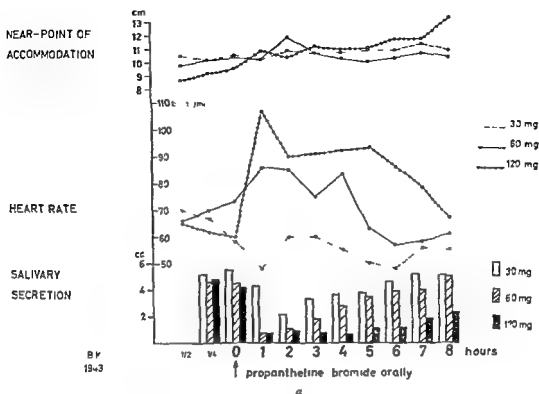


Fig. 4 (a) Anticholinergic effects of propantheline 30, 60 and 120 mg orally in subject B.K. (b) Anticholinergic effects of propantheline 3 and 10 mg i.m. in subject B.K.

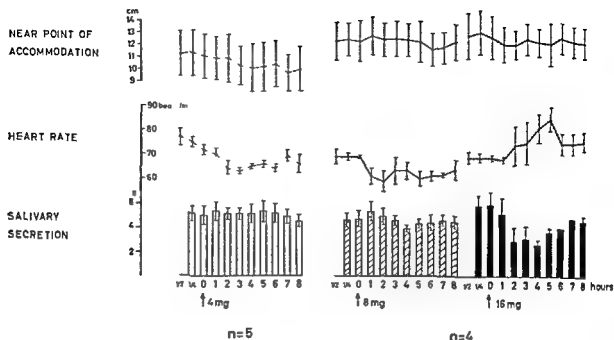


Fig 5 Anticholinergic effects of methylscopolamine 4 and 8 and 16 mg orally in four subjects (means \pm SE)

served apart from a moderate increase in heart rate in connection with lunch and a mild decrease in the accommodation of the eye during the seventh and eighth hour possibly due to fatigue.

For further evaluation of the method the effects of atropine were studied and found to be in accordance with earlier observations (2) and with the known fact that atropine is well absorbed from the gastrointestinal tract.

The ratio between the effective intramuscular and oral doses of propantheline was found to be approximately 1:10. There seem to be no earlier published studies comparing the effects of parenteral and oral propantheline in the same subject. The effective oral dose is similar to that previously observed by other authors describing effects on the salivary secretion (14) and the gastric acidity (6, 8, 10, 13).

The ratio between intramuscular and oral effective doses of methylscopolamine on salivation was found to be approximately 1:100. The minimum oral dose of methylscopolamine required to produce any effect on the studied parameters was about 8 mg. As 0.16 mg intramuscularly produced extensive effects lasting a couple of hours it is likely that less than 10 per cent of the dose administered orally was absorbed. Levine (9) showed that 10 to 30 per cent of methylscopolamine is absorbed from ligated intestinal loops in rats.

Investigations of cholinergic functions other than those studied here have also given results that indicate an incomplete absorption of methylscopolamine in the gastrointestinal tract. The ratio between the parenteral and oral effective dose of the compound on gastric secretion has been found to be 1:500 (6, 7). These authors have used the bromide salt of the compound while the nitrate was used in the present study.

The effects of parenteral butylscopolamine were found to differ both qualitatively and quantitatively from those caused by the other drugs. Firstly butylscopolamine caused effects of remarkably short duration. Secondly its effect on the near point of accommodation was extensive in a dose which produced only a moderate effect on salivary secretion. The other compounds were distinguished by more protracted effects and by a more extensive effect on the salivary secretion than on the other parameters studied. Such effects are considered to characterize anticholinergic drugs (4, 12).

The brief effect of butylscopolamine indicates

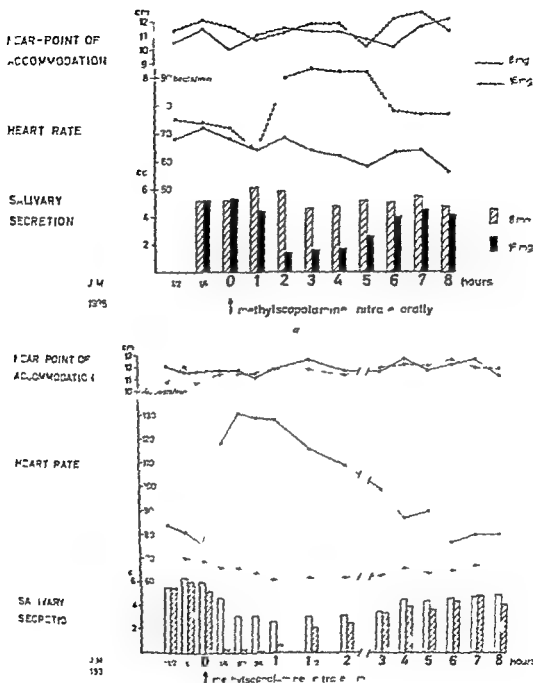


Fig 6 (a) Anticholinergic effects of methyloscopolamine 8 and 16 mg oral in subject J M (b) Anticholinergic

effects of methyloscopolamine 0.16 and 0.0 mg oral in subject J M

a rapid inactivation and/or a rapid excretion. Herberman and Hafels (5) estimated this to be about 20 mg per hour for a 70 kg adult, which is far from sufficient to explain the ineffectiveness of oral administration of 80 mg. Probably only

a very small amount of administered butyloscopolamine is absorbed in the gastrointestinal tract as has previously been pointed out (3, 5). The ineffectiveness of oral butyloscopolamine has also been demonstrated on gastric acidity and osmolarity

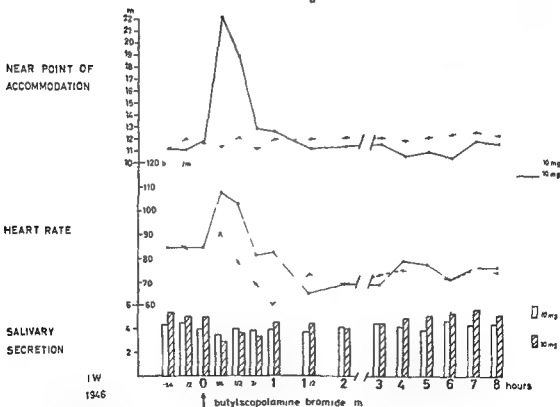
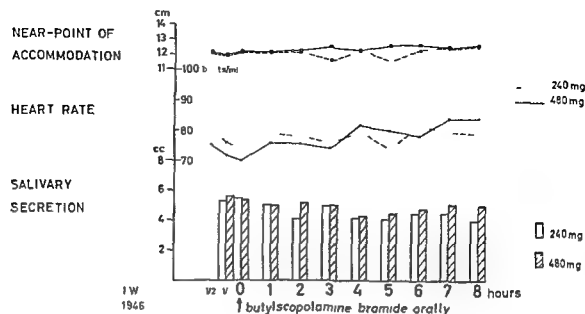


Fig 7 (a) The influence of butylscopolamine 240 and 480 mg orally on three cholinergic functions in subject I W 1946. (b) The influence of butylscopolamine 10 and 30 mg i.m. on three cholinergic functions in subject I W 1946.

and gastric evacuation rate. Thus 480 mg produced only slight effects on these parameters while small amounts of oral atropine and propantheline elicited extensive anticholinergic responses (1).

ACKNOWLEDGEMENTS

The present investigation was supported by the Sæd. b Medical Research Council (Project No B68 14X --7) and the Association of the Swedish Pharmaceutical Industry.

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ON THE PREVALENCE OF ADRENOCORTICAL ADENOMAS IN AN AUTOPSY MATERIAL IN RELATION TO HYPERTENSION AND DIABETES

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Abstract A prospective study was performed on an autopsy material consisting of 739 consecutive cases over 20 years of age (391 females 348 males) during a period of six months. The material included about 70% of the deaths in the city of Malmö (approximately 250 000 inhabitants). The adrenals were specially observed at autopsy for adenomas and hospital records were searched for hypertension and diabetes mellitus.

In the total material the frequency of adrenocortical adenomas was 8.7% which was considerably higher than previously reported figures. In patients with essential hypertension the adenoma frequency was 12.4% as against 7.9% in normotensives. This difference was not statistically significant. The adenoma frequency in men with secondary hypertension was remarkably high but the significance is not clear. In patients with hypertension the frequency of diabetes was 17.6% as compared to 9.0% in normotensives. This difference was statistically probably significant.

In patients with diabetes the frequency of adrenocortical adenomas was 16.5% in non-diabetics 7.7%. The difference was probably significant.

The syndrome of primary aldosteronism described by Conn (1) in 1955 is characterized by hypertension hypokalaemia and inappropriately increased production of aldosterone from an autonomous adrenocortical adenoma. Reports on verified cases with this syndrome show that these patients as a rule have had moderate hypertension with limited clinical manifestations for a long period of time whereas symptoms and signs of hypokalaemia have appeared rather late. The hypokalaemic symptoms often seem to have been the essential guide to correct diagnoses in most cases (5). The recognition of this fact has brought up the question of frequency of primary aldosteronism amongst patients with hypertension and it has been asked whether a significant number of patients with so-called essential hypertension in reality suffer from

primary aldosteronism with normokalaemia. Conn has advanced the hypothesis that 15-20% of all patients with benign essential hypertension have primary aldosteronism (3, 4). He arrived at these figures on the basis of earlier reports showing a higher frequency of adrenal adenomas in patients with hypertension (8, 9) as compared to normals. Furthermore Conn assumed that the frequent presence of decreased glucose tolerance amongst hypertensives (7) is caused by primary aldosteronism decreased glucose tolerance being found in about 50% of all cases (2). The present investigation was concerned with the prevalence of adrenocortical adenomas in relation to hypertension and diabetes mellitus in autopsy material from a random adult population.

MATERIAL AND METHODS

The studies were planned prospectively and included the autopsy material of Malmö General Hospital for six months February-July 1966. The material consisted of 739 patients, representing about 70% of the deaths within the city (total number of inhabitants about 250 000) during that period. Patients under the age of 20 were excluded but otherwise the material was unselected. The sex and age distribution is presented in Fig. 1.

Autopsies were performed according to the routine in the department of pathology. The adrenals were cut in mm wide slides and the presence of adenomas recorded. The diagnosis was mainly on macroscopic criteria. Adenomas varied from 2 mm to 4 cm in diameter. So-called nodular cortical hyperplasia was not included. The adenomas were studied microscopically (G. Ö.) and showed the same types of cells as the surrounding cortex.

In all 739 cases the hospital records were investigated (H. H.) with respect to the presence of hypertension or diabetes mellitus. A diagnosis of hypertension was based on repeated readings of a diastolic blood pressure above 100 mm Hg and electrocardiographic or roentgenologic

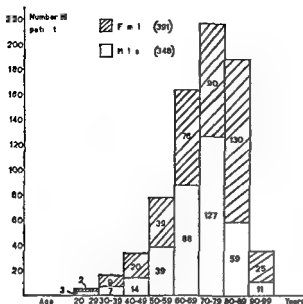


Fig 1 Sex and age distribution of the total autopsy material (739 patients)

signs of enlargement of the left ventricle or hypertrophy of the left ventricle at autopsy. Hypertension was divided into probably essential hypertension and so-called secondary hypertension, where there was evidence of renal disease. A diagnosis of diabetes mellitus was based on the presence of glycosuria and hyperglycemia $> 10 \text{ mg}\%$. The total number of diabetics was 79. In 76 the disease had presented after the age of 40. They had been treated with diet alone or in combination with sulphonyl urea or insulin.

RESULTS

Sixty-four cortical adenomas were found (Fig 2) representing a prevalence of 8.7%. In the females the prevalence was 7.2% and in the males 10.3% (Table I).

Hypertension was found in 119 patients and 620 were normotensive. In hypertensives the adenoma frequency was 12.4% as compared to

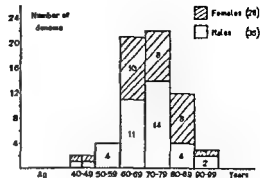


Fig 2 Distribution of adrenocortical adenomas according to sex and age in patients autopsied (391 females and 348 males)

7.9% in the normotensives. This difference was not statistically significant ($p > 0.2$).

In women with essential hypertension the adenoma frequency was 7.7% as compared to 7.4% in normotensives (Table I). Thus there was no positive correlation between the presence of hypertension and adrenocortical adenomas in women. In women with secondary hypertension no adenoma was found. In men with essential hypertension the adenoma frequency was 18.9% and 8.4% in normotensives; the difference is probably significant ($0.05 > p > 0.01$). In men with secondary hypertension the adenoma frequency was 26.7% which gave a clear statistical difference against normotensives (Table I).

Diabetes was present in 17.6% in hypertensives as compared to 9.0% in normotensive patients. The difference was probably significant, p being close to 0.01.

The adenoma frequency in diabetics was 16.5% in non-diabetics 7.7%. This difference was probably significant ($0.05 > p > 0.01$). In female diabetics the adenoma frequency was 16.3% in non-diabetics 5.8% ($0.05 > p > 0.01$). In male diabetics the adenoma frequency was 16.7% in non-

Table I Frequency of adrenocortical adenomas in relation to hypertension in 739 consecutive autopsies

Patients	391 Females			348 Males		
	With adenoma	Without adenoma	Frequency (%)	With adenoma	Without adenoma	Frequency (%)
Normotension	24	300	7.4	25	271	8.4
Ess. hypertension	4	48	7.7	7	30	18.9
Sec. hypertension	0	15	0	4	11	26.7
Total number	28	363	7.2	36	312	10.3

Table II Frequency of adrenocortical adenomas in relation to diabetes and hypertension in 739 consecutive autopsies

Patients	391 Females			348 Males		
	With adenoma	Without adenoma	Frequency (%)	With adenoma	Without adenoma	Frequency (%)
No diabetes	20	322	5.8	31	287	9.7
Diabetes: total number	8	41	16.3	5	25	16.7
Ess. hypertension + diabetes	1	12	7.7	1	2	33.3
Normotension + diabetes	7	23	23.3	3	23	11.5
Sec. hypertension + diabetes	0	6	0	1	11	(100)

diabetics 9.7% ($0.2 > p > 0.1$). Thus there was a probably significant difference in adenoma frequency in females but not in males.

The combination of adrenocortical adenoma, essential hypertension and diabetes was found in only two cases, giving a prevalence of 0.27% in the total material of 739 patients and 2.2% of all patients with essential hypertension. Adenoma in combination with secondary hypertension and diabetes was found in one case.

DISCUSSION

The frequency of adrenocortical adenomas in the present material was 8.7%. This figure is far higher than has been reported in some earlier investigations which showed a frequency of 1.4% (6, 8). These earlier investigations were retrospective studies whereas the present was prospective and it seems probable that a prospective study principally leads to a higher degree of accuracy. However, the frequency of adrenocortical adenomas in the Malmö autopsy material for the period 1957-1965 varied between 5.0 and 9.5% from one year to the other according to routine observations and records. There was no clear sex difference for that period and the adenoma predominance observed in men in the present material may be due to the rather limited number of cases.

Some earlier reports give a remarkably high frequency of adrenocortical adenomas in hypertensive patients. Thus Shamama et al. (9) found that the adenoma frequency was ten times higher in hypertensives as compared to normotensives and Russi et al. (8) found a fivefold increase. However, in a recent report no such difference was found (6). Our figures tend to support the latter

findings. In a study of the kind presented here the results are influenced by the criteria used to define hypertension. In the report by Russi et al. (8) the diagnosis of hypertension was based on heart weight exceeding 350 g and absence of myocardial or valvular disease. Heart configuration was not taken into account nor were the case histories investigated with respect to hypertension. In other studies (6, 9) similar criteria were used although clinical records were taken into account to some extent. We used more strict criteria in order to include only clinically significant hypertension (see above).

The high adenoma frequency in men with secondary hypertension and advanced renal damage is noteworthy. Even though the significance of this finding is not clear, a causal relationship is possible.

Our investigations hardly support the hypothesis advanced by Conn that primary aldosteronism could be present in about 15-20% of all cases with so-called essential hypertension. In our material the adenoma incidence was 12.4% in patients with essential hypertension (both sexes included) and this figure thus represents the highest possible relation of primary aldosteronism to essential hypertension. This figure should however be evaluated in view of the adenoma frequency found in normotensives, namely 7.9%. From this one might conclude that primary aldosteronism can be of importance in at most 45% of cases with clinically significant hypertension. However, we do not know whether the adenomas were aldosterone producing either in the cases classified as hypertensives or in the cases classified as normotensives. Thus the real role of the adenomas found in hypertensive patients cannot be established. The macro- and microscopic appearance of these

adenomas—whether found in hypertensives or normotensives—did not differ from that of aldosteronomas published earlier

Ostrander (7) observed an increased incidence of diabetes mellitus in patients with essential hypertension. This was to some extent supported by the present investigation. We found a high frequency of adrenocortical adenomas in the diabetic patients but the significance cannot be evaluated.

ACKNOWLEDGEMENT

This study was supported by a grant from the Swedish National Association against Heart and Chest Diseases.

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TISSUE AND PLASMA CORTISOL IN MAN UNDER VARIOUS CONDITIONS

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Abstract A recently developed micro-method for determination of cortisol in punch biopsy skin samples was applied to 158 patients with various diseases and drug treatments. Measurements of the total plasma concentration of cortisol were made simultaneously. The results seemed to reflect the actual cortisol content in the skin fairly accurately but considerable individual variations were present.

Prominent findings were the following. Treatment with oestrogen (as oral contraceptives) increased the plasma cortisol 2-fold with no concomitant change in the tissue concentration of cortisol. Women in the years around the menopausal age were frequently found to have a low tissue cortisol concentration with a normal plasma cortisol. Patients with connective tissue diseases including rheumatoid arthritis showed a non-significant elevation of the tissue cortisol but when such patients were treated with indomethacin the concentration of tissue cortisol fell significantly. In approximately 10% of all the patients the tissue/plasma ratio exceeded one. Three of these cases were treated with chloroquine. A moderate degree of positive correlation between plasma and tissue cortisol could only be demonstrated if factors believed to influence the distribution pattern of cortisol were avoided. On the basis of these observations it is suggested that other mechanisms, in addition to a shift in the proportion of cortisol binding to plasma proteins affect the cortisol distribution in the body. Protein binding within the tissue is discussed as a possibility.

Except for the adrenal cortex, precise information about the concentration of cortisol in tissues from human beings does not seem to be available. However, the relationship between plasma and tissue concentrations of cortisol in patients with various diseases, hormonal disorders and in different drug treatments are of interest as shifts in free and bound forms of plasma cortisol may not be the only mechanisms by which this hormone in the tissues could be influenced. A chemical rather than a radioisotopic technique should be preferred when dealing with human beings, al-

though static observations may have limited interpretations.

The present study deals with the problem outlined above by using a recently developed micro-method (6) by which cortisol is spectrophotofluorometrically determined in small tissue samples obtained by punch biopsies. General information concerning the usefulness of this approach has been the immediate objective rather than the underlying biological problems.

MATERIAL AND METHODS

Material

One hundred and fifty-eight patients (89 women and 69 men) from ages 16 to 78 years were studied (Table I). All patients were selected from a general medical ward. Approximately one third were chosen with the intention of studying special conditions. Although the remaining patients were chosen at random, some categories have been deliberately avoided. These categories involved patients failing to give their consent, very obese patients, patients in acute and/or life threatening situations and patients with severe diabetes mellitus. The majority of the patients were not seriously ill. Sampling with controls was not performed in the usual manner. However, a reasonably good distribution in respect of sex, age and various diseases was achieved within the limits of the present material.

Sampling procedure

The sampling was constantly performed at approximately 7.30 a.m. in order to avoid diurnal variations as much as possible. Tissue samples were obtained from normal skin by punch biopsy in the gluteal region after local analgesia with ethylene dichloride. A biopsy punch of 6 mm in diameter was used and bleeding was most unusual. The weight of the tissue sample was usually in the range of 30-70 mg. Weighing, wrapping in aluminium foil and storing at -25°C were carried out immediately after the tissue removal. The blood samples, using heparinized tubes, were drawn from the cubital vein 15 min

Table I Patient material

Group	Number of pat		
	Females	Males	Total
Adrenal or pituitary disorders	8	5	13
Treated with ACTH or glucocorticosteroids	7	8	15
Dysfunction of sexual hormones or treated with such	15	—	15
Collagen diseases incl rheumatoid arthr. Not treated with glucocorticosteroids	22	7	29
Various other patients	37	49	86
Total material	89	69	158

mediately before the biopsy procedure. Separation and storing of the plasma at -20°C was done within minutes after the blood was drawn. The only exceptions from the above described procedure were two patients in whom ACTH tests were performed in the evening. Tissue and blood samples were obtained just before and immediately after a 4 hour i.v. infusion of 70 units ACTH. The second punch biopsy was done in the opposite gluteal regions.

Analytical method

The analytical procedure was initiated within a few hours after the sampling simultaneously for plasma and tissue. Essentially the analytical procedure (6) is a micro scale method based on a spectrophotofluorometric technique. It

allows for reasonable precision and accuracy in determining cortisol down to about one nanogram per sample. The specificity is high and corticosterone is quantitatively removed during the extraction procedure. The results are given as concentrations in ng/ml plasma and ng/g wet weight tissue respectively.

RESULTS

The biological validity of the method was tested in a limited number of patients in whom the results could be logically predicted. Table II demonstrates a series of patients treated with ACTH or prednisone and Table III shows the results from patients known to suffer from either deficiency or overproduction of adreno-cortical steroid hormones. In general the results are compatible with what could be expected: low values in varying degree of adreno-cortical suppression with prednisone and in Addison's disease and high values in Cushing's disease or intensive cortisone treatment. However considerable individual variations exist. The results from Tables II and III seem to indicate that the tissue concentrations of cortisol determined by this method are reliable and specific to an extent to which they can be used for investigative purposes.

Although no attempt has been made to measure

Table II The concentration of cortisol in plasma and skin in patients treated with ACTH or glucocorticosteroids

Sex	Age	Diagnosis	Medication (daily dose) and comments	Conc of cortisol	
				Plasma (ng/ml)	Tissue (ng/g)
o	57	Cancer coli metast	Meprobamate aspirin codein	(i) 33 ^a	30
				(ii) 43 ^a	740
♂	38	Cardiospasm	0	(i) 53 ^b	8
				(ii) 29 ^b	99
o	25	Compt vert thorac	Adreno-cortical supp test	0	—
♂	68	Panart nodosa	Prednisone 25 mg Imuran	24	35
♀	62	Autoimmune hemolyt anemia	Prednisone 20 mg	0	0
—	36	Urticaria arthralgia	Prednisone 20 mg (four days) cyproheptadine	160	70
♀	47	Sarcoidosis	Prednisone 17.5 mg	29	0
♀	41	Pulmonary fibrosis	Prednisone 17.5 mg	0	0
♀	24	Hodgkin's disease	Prednisone 15 mg, cyclophosphamide	13	55
o	—	Pneumonia asthma pern anemia	Prednisone 15 mg, digitalis penicillin chlorthalidopride	0	74
♀	—	S.L.E.	Prednisone 10 mg indomethacin chloroquine	10	65
o	43	Bronchial asthma	Prednisone 10 mg theophyllamine	77	118
♀	58	Bronchial asthma bronchitis	Prednisone 7.5 mg	41	4
♀	2	Bronchial asthma bronchitis	Prednisone (disc 12 days previously)	101	31
—	65	Bronchitis	Ledercort (disc 16 days previously)	133	101

^a Between (i) and (ii) an i.v. infusion was given containing 70 units ACTH (4 h)

^b As the patient above

^c Four days administration of dexamethasone

Table III The concentration of cortisol in plasma and skin in patients with present or previous disorders in the hypophysis or the adrenal cortex

Sex	Age	Diagnosis	Medication (daily dose) and comments	Conc of cortisol	
				Plasma (ng/ml)	Tissue (ng/g)
♀	55	Addison's dis	Cortisone 50 mg Hydrocortisone 0.1 mg No cortisone for 3½ days	0	0
♀	71	Addison's dis	No treatment	0.5	0
♀	43	Addison's dis	Cortisone 25 mg Hydrocortisone 0.1 mg No cortisone for 24 hours	0	190
♂	19	1 Addison's dis diabetes	Insulin	53	93
♀	64	Hypophysectomized	Cortisone 150 mg thyroxine penicillin Adrenal crisis two weeks ago	227	179
♂	37	Cushing's dis	Hydralazine thiazide	380	76
♀	41	Previously treated Cushing's dis	(i) Cortisone 12.5 mg (ii) No cortisone for 12 days	(i) 110 (ii) 136	83 36
♀	64	Previous Cushing's dis	Guanethidine thiazide Clinically normal	160	23
♂	73	Previous Cushing's dis	Papaverin Clinically normal	155	58
♂	38	Previous Cushing's dis	0 Clinically normal	137	65
♀	45	Previous Cushing's dis	Trusil thiazide Clinically normal	146	61
♂	37	Adrenal steroid metabolism defect	0 Huge excretion of 17 keto-steroids Clinically normal	130	56
♂	15	Hypophysis gigantismus	X ray on the hypophysis	130	52

the different plasma fractions of cortisol a positive correlation between the corresponding values for total concentrations in plasma and in tissue should be readily expected. A highly selected group of patients was chosen to avoid the influence of "irrelevant" factors (see later). Thus the corresponding values shown in Fig. 1 derive from 48 male patients not suffering from endocrine or collagen diseases nor treated with hormones. In spite of considerable individual variation a medium grade of positive correlation can be demonstrated ($r = 0.443$). In contrast an attempt to correlate the tissue/plasma ratio to the age in the same group gave a negative result (Fig. 2).

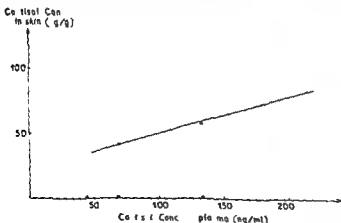


Fig. 1 The concentration of cortisol in skin (ng/g) is plotted against the plasma cortisol concentration (ng/ml) for 48 male patients not suffering from endocrine disorders or collagen diseases nor treated with hormones. A medium grade of positive correlation was found (regression coefficient = 0.443). The regression line is $y = 1.43 + 0.285x$.

The average values for 81 patients (48 men and 33 women) are given in Table IV. The group includes all ages and a variety of diseases and treatments but excludes patients with diseases in the adrenal cortex, the hypophysis, the connective tissue or the reproductive organs as well as patients undergoing hormonal therapy. The average results for plasma and tissue cortisol are slightly but non-significantly higher for the females.

The possible influence of oestrogen has been illustrated in Table V. Treatment with oral contraceptives (and thus oestrogens) raised the plasma concentration about 2½ fold while the tissue concentrations remained unchanged. Thirty three

Table IV The concentrations of cortisol in plasma and skin in patients without collagen diseases dysfunction in the sexual or adreno-cortical hormone systems nor undergoing hormonal treatment

Sex	Age range	No of pat	Plasma (ng/ml) \pm s.d.	Tissue (ng/g) \pm s.d.
♂	16-78	48	117.1 \pm 43.1	54.9 \pm 27.3
♀	16-69	33	130.1 \pm 4.7	60.2 \pm 41.3
♂ + ♀		81	122.4 \pm 43.4	57.0 \pm 33.8

Table V Relation of oestrogenic influence to the concentration of cortisol in plasma and skin in women

Group	No of pat	Plasma (ng/ml) \pm s.d.	Tissue (ng/g) \pm s.d.
Treated with oral contracept. Age 20-41 years	8	308.9 \pm 96.9 ^a	65.5 \pm 26.7
Thirty three female patients from Table IV			
I 16-40 years No hormones	9	133.4 \pm 49.7 ^a	75.6 \pm 46.2 ^b
II >40 years either still menstr. or menopause < 2 years	11	127.8 \pm 31.2	50.9 \pm 49.2 ^b
III Menopause > 2 years	13	129.1 \pm 49.1	58.2 \pm 30.2 ^b

^a The difference is statistically significant at $p < 0.01$

^b The difference is not significant (Duncan's multiple range test). Compare Fig. 3

Table VI The concentration of cortisol in plasma and skin in five women with dysfunction in the sexual hormone secretion or in treatment with sex hormones

Age	Diagnosis and comments	Conc. of cortisol	
		Plasma (ng/ml)	Tissue (ng/g)
24	? S.L.E. Receives oral contraceptives (not been taken for 6 days)	89	83
59	Irritable colon and climacterium Oestrogen inj. every 4th week. Last inj. 3 days previously	190	7.
24	Pregnant in the 7th month	155	30
24	Anorexia nervosa Menostasia for 2 years. Chlorpromazine 150 mg daily	116	0
28	Stein Leventhal's syndrome Migraine medication dihydro-ergotamine	101	37

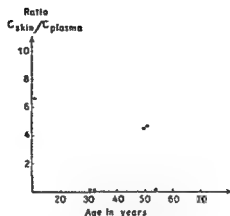


Fig. 4. For the same 48 male patients as in Fig. 1 the ratio between the cortisol concentration in skin and in plasma is plotted against age. No correlation could be demonstrated.

women were divided into three arbitrary groups supposedly reflecting different levels of endogenous oestrogen production. No difference in the plasma values was noted among the groups but a tendency to lower concentrations of tissue cortisol in the years around the menopausal age seems clear. Because of the large individual variations the difference is not statistically significant in this small number of patients (Duncan's test at the 5% level). Fig. 3 shows the individual plottings of tissue/plasma ratios for the three groups. As can be seen 8 out of 11 patients in the "menopausal" group showed exceptionally low values. In Table VI an attempt to illustrate the influence of oestrogen on the distribution of cortisol has

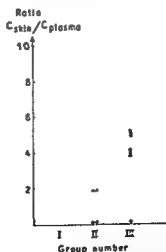


Fig. 3. For 33 females the ratio cortisol in skin (ng/g)/cortisol in plasma (ng/ml) is shown for group I < 40 years, group II > 40 years either still menstrating or menopause for < 2 years and group III menopause > 2 years.

Table VII The concentrations of cortisol in plasma and skin in various groups of patients

Groups	No of pat	Plasma (ng/ml) \pm S.D.	Tissue (ng/g) \pm S.D.
Various diseases and drugs totally (from Table IV)	81	122.4 \pm 43.4	57.0 \pm 33.8 ^a
Special groups			
Hepatic cirrhosis	6	146.8 \pm 60.5	108.5 \pm 46.0 ^a
G.I. dis.	12	131.4 \pm 49.6	47.0 \pm 43.0
Cardiovascular dis	12	112.0 \pm 48.0	55.0 \pm 30.7
Renal and urinary tract dis	10	128.1 \pm 47.8	65.9 \pm 32.5
Hematological dis	7	110.3 \pm 48.1	41.0 \pm 34.3
Pulmonary dis	9	106.3 \pm 36.5	48.1 \pm 33.4
Non rheumatic joint dis	8	130.3 \pm 5.2	49.0 \pm 20.6
Chlordiazepoxide or diazepam	11	134.3 \pm 37.2	48.6 \pm 37.8
Various anti psychotic drugs	12	123.4 \pm 28.3	64.9 \pm 30.5
Collagen diseases including R.A.	21	136.2 \pm 55.7	78.2 \pm 44.0 ^a
Indomethacin 75-125 mg daily	9	92.2 \pm 31.1	36.6 \pm 34.1 ^a

^a The difference is statistically significant $p < 0.05$

been made by presenting five female patients in various hormonal conditions. These patients are listed separately as they are not included in other groups.

In Table VII all 31 patients from Table IV are grouped according to the organ or system in which their actual disease is diagnosed. No concern is paid to clinical entities, age, sex or therapy. Only special groups large enough to be representative are listed, but some overlapping exists. Two groups of drug treatments are added as many patients did not in fact receive drugs at the time of the biopsy. No significant correlation seems to exist between this crude classification and the observed cortisol concentrations. The only exception is a small group of patients with hepatic cirrhosis whose tissue concentration of cortisol was significantly higher than that of the rest of the group. A separate group of 21 patients with various collagen diseases (including rheumatoid arthritis) had on the average higher tissue levels than patients with out collagen diseases, but the difference is not significant. The comparability of the two groups may be questioned, but the group with collagen diseases as would be expected, was not biased by

Table VIII Patients having a higher concentration of cortisol in the skin than in the plasma (see text for details)

Sex	Age	Diagnosis	Medication and comments	Conc. of cortisol	
				Plasma (n./ml)	Tissue (ng/g)
♂	69	Bronchial asthma, pneumonia, pern. anemia	Prednisone 15 mg, digitalis, penicillin	0	24
♀	43	Addison's disease	Chlordiazepoxide Cortisone 25 mg, fludrocortisone 0.1 mg	III	190
♀	38	SLE	No cortisone for 24 h Prednisone 10 mg, indomethacin, chloroquine	10	65
♂	68	Panarteritis nod.	~50 mg Prednisone 25 mg, Imuran	~4	33
♀	38	Bronchial asthma	Prednisone 7.5 mg	41	42
♀	40	Prim. amenorrhea	0	49	92
♂	19	Addison's disease, diabetes	Insulin	53	93
♂	43	Bronchial asthma	Prednisone 10 mg, theophylline	77	108
♀	51	Mb. cord. nervousism	Chlordiazepoxide, stemetil, digoxine	III	171
♀	56	Bronchial asthma	0	91	101
♀	21	Observatio. obese	0	97	115
♂	56	Hepatic cirrhosis	0	57	146
♀	19	Pubertas tarda	Diazepam	III	285
♀	37	Rheumatoid arthr.	Truxal, chloroquine 50 mg	150	1038
♀	17	Urticaria	0	150	273
♀	63	Paraproteinemia	0	155	790
♀	73	SLE	Chloroquine 250 mg	173	204

Table IX The concentration of cortisol in plasma and skin in five patients receiving treatment with chloroquine

Sex	Age	Diagnosis	Medication	Plasma (ng/ml)	Tissue (ng/g)
♀	34	SLE	Chloroquine 250 mg Prednisone 10 mg Indomethacin 50 mg	10	65
♀	32	Rheum arthr	Chloroquine 250 mg Truxal 45 mg	150	1038
♀	73	SLE	Chloroquine 250 mg	173	204
♀	40	Hemolyt anemia Hypothyroidism	Chloroquine 250 mg Thyroidin 1.0 mg	165	0
♀	41	SLE	Chloroquine 250 mg	101	65

an over representation of young women which would have influenced the average cortisol level. The treatment with indomethacin is accompanied by a statistically significant fall in the tissue cortisol when compared to the group of untreated cases of connective tissue diseases. However the correctness of such a comparison may be disputed from both a statistical and a biological stand point.

A set of observations not fully understood is listed in Table VIII. In 17 out of 158 patients a higher concentration was found in tissue (per gram wet weight) than in plasma (per ml). These patients are not incorporated in other groups. In a majority of the 17 cases with reversed ratio the differences between plasma and tissue values are not very large but in a few cases considerable. Analytical errors are not likely but unfortunately re biopsies were not performed. Three out of the 17 patients were being treated with chloroquine and therefore five patients receiving this drug (of the total 158 patients) are listed individually in Table IX. Investigations in our laboratory have confirmed that chloroquine as such does not interfere with the determination of cortisol in the used analytical procedure. However it is not known whether this is the case for any of the four or five metabolites chloroquine is known to produce.

DISCUSSION

Application of the method

Determinations of cortisol other than in the adrenal cortex and plasma are few in number and radio isotopic methods have been preferred (2, 4, 8, 12). Actual tissue concentrations of cortisol in humans performed by chemical determinations do not seem to have been studied previously.

Consequently the results from the present investigation cannot be compared directly with others but from the results presented in Tables II and III it seems reasonably acceptable that the method is capable of reflecting the actual content of cortisol in the tissue sample. The applicability of the method for correct determination of plasma levels is obvious. It is however a matter of dispute whether the figures for the tissue given as a concentration of cortisol in the total tissue is a biologically correct expression and whether or not this can be compared to the plasma concentration. Reasons for this possible limitation are that the distribution space for cortisol is in fact not defined for the tissue (skin and subcutaneous tissue) used in this investigation. Furthermore as pointed out by Plager et al (1) the form in which cortisol exerts its physiological activities in the tissues is not known and it should be added that the concentration—at tissue level—necessary for its normal activity is also unknown. Finally the present method like many others gives no information concerning the rate of turnover of cortisol in the tissue for which reason conclusions based upon tissue concentrations alone could be grossly misleading with respect to the dynamics of this hormone.

The influence of oestrogen

Previous studies of the influence of drugs and diseases on the distribution pattern of cortisol in the body have predominantly been approached from the point of view of plasma protein binding. The influence of oestrogen has attracted considerable interest after the observation was made that oestrogens sharply increased the plasma cortisol concentration (2, 4, 11, 13, 20, 21). It is a remarkable characteristic that clinical evidence of hypercorticism is not present in these patients.

(9-10) These patients are found to have an increased transcortin bound fraction of plasma cortisol as well as transcortin itself. However according to several investigators the free cortisol remains unchanged (14-15, 17-18, 20). It is therefore usually assumed that the tissue concentration of cortisol also remains unchanged as only free plasma cortisol is believed to be in equilibrium with tissue cortisol. However the oestrogen-cortisol relationships may be more complex than implied in the above cited works as stressed by Plager et al (11) who also found the free fraction of plasma cortisol increased during oestrogen treatment while the rate of cortisol production was unchanged. Furthermore Sandberg et al (15) have stated that about 50% of the total amount of transcortin is located outside the vascular bed. To what extent cortisol is bound to this portion of transcortin remains to be clarified but only non protein bound cortisol is physiologically active in the tissue (1-7). Bradley and Waterhouse (2) confirmed in patients that the total extravascular pool of cortisol was unaffected by oestrogen while the intravascular concentration was increased significantly. As ACTH stimulation increased both they concluded that the kinetics of cortisol was changed by oestrogen consistent with an increase in the transcortin level. The actual extravascular concentration could not be calculated because the distribution space was unknown.

The results from the present investigation confirm that the total plasma concentration of cortisol is increased during the treatment with oral contraceptives (and thus oestrogens) but that the concentration of tissue cortisol is unaffected. Hence direct tissue determinations are in accordance with the previous observations which show that conditions with elevated oestrogen affect the distribution pattern of cortisol in the body. As no distinction was made between bound and free plasma cortisol the relative shift in these fractions was not recorded in our study. However the results seem to indicate that the net movement of cortisol from plasma to tissue is unaltered providing an unchanged turnover of cortisol in the tissue.

In order to study the possible influence of physiological variations of oestrogen simultaneous oestrogen and cortisol determinations should ideally be performed. Such results are not available. The material in the present investigation was too small for an attempt to relate the con-

centrations of plasma and tissue cortisol in younger women to the time in the menstrual cycle. Another approach was made by dividing 33 women into three arbitrary groups hopefully reflecting different levels of oestrogen. The younger females presumed to have a higher average oestrogen production also had the highest concentration of cortisol in the tissue whereas the women centered around the menopausal age showed the lowest average concentration. The plasma levels were similar in the three groups but Fig. 3 clearly demonstrates that the majority of low tissue/plasma ratios are found in the years around menopause. A similar variation was not seen in males (Fig. 2). These observations although grouped slightly differently were published in a preliminary report (16). The results strongly suggest that the distribution of cortisol in females is also influenced by the endogenous oestrogen production. This observation is however difficult to explain only on the basis of a shift in the free and bound portions of plasma cortisol. If this were the case a relatively larger part of the total plasma cortisol should be bound to protein at a lower oestrogen level which is unlikely. The decrease in tissue cortisol in women around the menopausal age is not associated with any clinical manifestation of hypocorticism indicating no change in the physiological active cortisol. It might therefore be suggested that cortisol exists in the tissue in at least two different forms one of which is inactive and presumably bound to a protein. Speculatively a temporary decrease in such a cortisol binding protein in the tissue could be caused by the lack of oestrogenic stimulation on the protein production resulting in a decrease in the total tissue concentration of cortisol with no change in the cortisol activity.

Connective tissue disorders

The rheumatic diseases may possibly alter the metabolism and distribution of corticosteroids in the connective tissue although most investigators have failed to clearly demonstrate such a relationship (5-22). The present investigation suggests a tendency to higher tissue concentration of cortisol in patients with collagen diseases and rheumatoid arthritis. This tendency however is not significant but the decrease in tissue cortisol observed after the treatment with indomethacin in such cases is striking. Indomethacin was the only

non steroid anti inflammatory agent studied and although the findings seem to be fairly consistent much more work needs to be done to confirm this observation. Brodie (3) suggested on the basis of corticosterone determinations in rats that non steroid and anti inflammatory drugs exceeded their activities by an increase of the unbound plasma steroid and therefore indirectly raised the tissue concentration of the active hormone. Our results do not support this hypothesis. However as nothing is known about indomethacin's influence on the metabolism of cortisol in the tissues an indirect action of this agent via steroids cannot be completely ruled out. The results presented in this study do suggest a relationship between the action of indomethacin and the distribution of cortisol. Alterations in the properties of tissue binding of cortisol might also be suggested.

Other conditions

A relatively impaired steroid metabolism in the liver may be a reasonable explanation of the higher concentration of cortisol found in the skin of patients with cirrhotic livers but the number of patients is too small for any definite conclusion. More attention should be directed to the finding that approximately one tenth of the total material showed a higher concentration of cortisol in the tissue than in the plasma. About two thirds of these cases may be seen in the light of their special hormonal conditions or some may even be explained on the basis of experimental variations as the difference between the tissue and plasma values is not very large. A group of six patients in which the reverse difference is considerable is not easily explained. It confirms the impression however that cortisol (evidently in an inactive form) can exist in a high concentration in the tissue with no relation to the simultaneous lower plasma concentration. The observation that three such reverse ratios were found among totally five patients treated with chloroquine may suggest a connection between this agent and a peculiar tissue binding of cortisol.

The discrepancy between corresponding plasma and tissue concentrations is a conspicuous finding in the present material. A moderate degree of positive correlation can only be demonstrated when many factors believed to influence the distribution of cortisol are excluded (Fig. 1). In fact the observations from the entire material and the special

examples discussed above leave the impression that the two sets of simultaneous determinations cannot really be related to each other. Relative changes in the free and protein bound plasma cortisol seem only to a certain degree to explain the discrepancy so that additional factors must be present. Only about 10% of the plasma cortisol is free and diffusible (20). On the average however tissue cortisol is found to be about 50% of the plasma concentration. The difference between the 10% diffusible cortisol and the 50% actually observed in the tissue cannot be explained only on a basis of different distribution spaces. This also points to the possibility of more factors involved in the distribution of cortisol than the plasma protein binding. Some of the results suggest that the variations in the tissue concentration are time related a phenomenon well known for plasma but the tissue variation might be out of phase with the fluctuations in the plasma concentration. If so cortisol can be retained in the tissue in spite of a decreasing plasma concentration. The observations made in the present investigation taken in conjunction with the results of Plager et al. (11) therefore suggest that plasma and tissue concentrations of cortisol may vary partly independently of each other. Undoubtedly the tissue cortisol is supplied by the circulating pool of the hormone and the active concentration of tissue cortisol can be increased rapidly from the free plasma cortisol. But other slower acting mechanisms also seem to influence the distribution pattern. Such mechanisms may be located in the tissue and they seem to influence the distribution of cortisol within the tissue as well as across the vascular membrane without necessarily or primarily affecting the biological activities of the hormone.

ACKNOWLEDGEMENTS

This investigation has been supported by grants from the Danish State Research Foundation (no. 133/66 and no. 131/67) and the Daell Foundation, Copenhagen.

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SYSTEMIC ARTERIAL HYPERTENSION

Aspects of Etiology and Pathogenesis in a Retrospective Study of a Hospital Material

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Abstract Among 301 cases of unselected hypertension 45% proved to be of the essential and the remaining 55% of the secondary type. A sex difference was present in that the essential type was found in 53% among men against 33% among women. The major causes of secondary hypertension were chronic pyelonephritis and nephritis, Cushing's disease and allied disorders and aortic coarctation. In a series of 177 patients with selected hypertension 76% maintained the diagnosis of essential hypertension. No significant sex difference was observed. All the cases of secondary hypertension were of renal origin, chronic pyelonephritis being responsible for more than 80% of the cases. Of the techniques employed urography with early sequential exposures seemed to be the method of choice for screening purposes. Renal biopsy was similarly helpful in obtaining the correct diagnosis. Renal angiography also proved to be of great significance and its use should clearly be extended. The presently available methods for evaluating systemic arterial hypertension are discussed.

Recent advances in methods of study have served to clarify the etiology of systemic arterial hypertension. Secondary hypertension in which the cause of the blood pressure elevation can be demonstrated has increased while the so-called essential hypertension has proportionately declined.

Although this is widely recognized the extent of this change is little known.

The present study provides information on the etiology of arterial hypertension as obtained in a department with access to most modern methods of investigation.

The study has been conducted along two lines. Firstly an analysis has been made of the total number of patients who during the observation period had sustained hypertension regardless of their main disease. Secondly a similar analysis has been carried out of those patients who were exclusively admitted for evaluation of systemic

arterial hypertension. The study thus comprises unselected as well as selected cases of hypertension.

MATERIAL

Included in the study were all patients with sustained hypertension and with blood pressure above 150/100 mm Hg and who were admitted during 1965-1966 and the first five months of 1967.

Ages ranged from 15 to 69 years with an identical mean age of 51 years for both sexes.

A great number of the patients were referred from other hospital for further evaluation in a University clinic. So far the material is to be regarded as selected.

METHODS

In addition to routine clinical examinations the patients were investigated by means of urography with early sequential exposures (9), radioisotope renography and when necessary renal angiography and renal biopsy. Differential renal function tests were not consistently carried out. Angiotensin infusion tests were not at all employed due to their doubtful significance (7). The department had no access to methods for estimation of the pressor substances renin and angiotensin. Primary aldosteronism was searched for by ordinary electrolyte studies and by assessing urinary output of aldosterone. In the majority of the cases catecholamines and steroids were estimated in the urine.

Essential hypertension was classified according to the system suggested by Rasmussen (14). Following this system the malignant type is characterized by the presence of papilledema while the levus type shows only minor retinal vascular changes and no damage of the target organs. In between these two types the gravis type is situated.

RESULTS

It can be observed that less than half of all the patients suffered from the essential type of hyper-

Table I The series of unselected arterial hypertension and its distribution in subgroups

		Essential		Secondary	
		No		No	
Men	172	91	53	81	47
Women	129	43	33	86	67
Total	301	134	45	167	55

Table II The underlying diseases of secondary hypertension

	Men	Women
Chronic nephritis	38	17
Chronic pyelonephritis	8	18
Cushing's disease	7	14
Aortic coarctation	7	11
Renal artery stenosis	7	2
Pheochromocytoma	1	1
Hyperparathyroidism	0	2
Varia	13	21
Total	81	86

Table III The series of selected arterial hypertension and its distribution in subgroups

		Essential		Renal	
		No		No	
Men	115	91	80	24	20
Women	62	43	70	19	30
Total	177	134	76	43	24

tension (Table I). Among women only one third had this type.

As expected chronic nephritis and pyelonephritis were responsible for a major part of the secondary types of hypertension (Table II). The sex linked preponderance of these disorders is clearly illustrated. Reservations have to be made for the many cases of Cushing's disease. Only one third were new cases, another third were cases admitted for control, whereas the remainder consisted of cases in which the diagnosis was under suspicion. Aortic coarctation is also over represented in the series, a mere consequence of the fact that the department is mainly occupied with cardiovascular disease. Pheochromocytoma was only found in less than 1%. Being a curable form

Table IV The underlying diseases in renal hypertension

		Chronic pyelo-nephritis		Chronic nephritis		Renal artery stenosis	
Men	25	8	10	7			
Women	18	14	2	2			
Total	43	22	12	9			

of hypertension, however, its detection remains essential. A considerable amount of the secondary hypertension (*varia*) comprised collagen disorders, coronary and valvular heart disease, peripheral vascular disorders and diabetes mellitus. There were three cases of polyarteritis nodosa. Not a single case was encountered of primary aldosteronism or bilateral cystic disease of the kidneys.

A total of 177 patients were admitted exclusively for evaluation of systemic arterial hypertension (Table III). The vast majority were men. A careful study of all these patients gave as a result that 76% maintained the diagnosis of essential hypertension, the remaining 24% being hypertension of renal origin.

A majority of 64% of the essential hypertension cases were of the gravis type, whereas only 6% fulfilled the criteria for malignant hypertension (14).

In renal hypertension, chronic pyelonephritis most often was the underlying disease, accounting for 51% of the cases (Table IV). Chronic nephritis and renal artery stenosis were responsible for 29% and 10% respectively. For the two first mentioned disorders, the typical sex-linked preponderance is clearly shown. If related to the whole series of 177 patients, the three renal disorders above were responsible for 12.7% and 5.6% of the cases.

Table V The total number and the results of investigations undertaken in 170 patients with selected arterial hypertension

	Total	Pathol.		Failures	
Urography	115	68	47	16	14
Renography	123	72	77	31	25
Renal angiography	68	40	42	62	
Renal biopsy	23	14	20	87	

of arterial hypertension. An extended use of renal angiography would certainly have produced an increase of hypertension due to renal artery stenosis.

Seven patients were adequately examined prior to the admission. The number and the results of the various investigations undertaken in the remaining 170 patients are presented (Table V). Urography was only conducted in two thirds of the patient group. This was due to omission of this examination in nearly all the patients having the levis type of hypertension. As both chronic pyelonephritis and renal artery stenosis can exist with normal urinary findings and with blood pressure falling to normal values during hospitalization it is mandatory that urography be carried out in all cases of hypertension.

Renal angiography was undertaken in only 40% of the patients. Probably a more frequent use of this method would have brought forward a greater number of renal hypertension.

Urography as well as renography provided several erroneous results: urography by missing positive findings; renography by detecting far too many cases with apparently significant reduction of renal function and renal blood flow.

Disparity of the length and size of the kidneys was a consistent finding in chronic pyelonephritis whereas such disparity was present in only four out of nine cases of renal artery stenosis (Table VI). Aplasia of one kidney was found in five cases by means of urography. Subsequent renal angiography proved two cases of aplasia to be due to complete stenosis of the ipsilateral renal artery (Table VII). The condition known as fibromuscular hyperplasia was found to be present in the renal artery of a woman. Systolic bruits over the actual renal artery were heard in less than half the cases of renal artery stenosis.

Of 23 renal biopsies three were unsuccessful. Of the remaining biopsies 17 were undertaken for diagnostic purposes only (Table VIII). The questioned diagnoses naturally were chronic nephritis and pyelonephritis, sarcoidosis and amyloidosis. In ten instances biopsy was undertaken of the diseased or most affected kidney and in seven instances of the presumably healthy one. The latter were carried out to determine operability. For the same reason biopsy of the contralateral kidney was made in three instances when unilateral renal artery stenosis was present.

Table VI Findings in 47 urographies with pathological result

Small kidney on one side	10
Small kidney on both sides	3
Renal cyst	3
Stone	2
Tumor	5
Aplasia of one kidney	5
Delayed "appearance time" on one side	5
Delayed "appearance time" on both sides	5
Anomalies of calyces pelvis ureter	9

Table VII Findings in 42 renal angiographies with pathological result

Renal artery stenosis on one side	6
Renal artery stenosis on both sides	6
Renal artery occlusion on one side	1
Fibromuscular hyperplasia of one renal artery	3
Aplasia of one kidney	2
Renal artery aneurysm on one side	1
Atherosclerosis of abdominal aorta	4
Renal cyst	10
Small kidney on one side	3
Small kidney on both sides	

Table VIII Findings in 20 renal biopsies with pathological result

Chronic nephritis	2
Chronic pyelonephritis	1
Renal arteriosclerosis	10
Renal arteriosclerosis	1

The biopsy specimens most often provided the correct diagnosis. However a great number only showed arteriosclerosis compatible with hypertension but without specified diagnosis. A majority of these specimens belonged to kidneys with otherwise clear signs of chronic pyelonephritis. Knowledge of clinical data therefore seems necessary for correct interpretation.

In the three biopsies from the contralateral kidney in patients with renal artery stenosis on one side significant arteriolar damage was found. Consequently no surgery was undertaken. In two patients biopsy was done on both kidneys first for diagnosis thereafter to determine operability. No serious complications followed the performance of biopsy of the kidneys in the present series.

A study was also made of age, duration of the hypertension, its acceleration, renal function and electrolytes. No significant differences in these respects were found between the various types of hypertension. Naturally the target organ damage

corresponded with the severity of the disease. Hypocalcemia similarly occurred more often in severe hypertension both spontaneously and as a result of treatment with diuretics.

Although regarded as unspecific the symptoms produced by hypertension are unquestionable. More than two thirds of the patients with essential hypertension had subjective complaints. Usually these symptoms were relieved by adequate lowering of the blood pressure. Symptomatology was of the same order and nearly of the same severity in both severe and mild hypertension but notable breathlessness and tiredness always signaled serious hypertension.

COMMENTS

Recent studies have shown that systemic arterial hypertension even in mild form bears a gloomy long term prognosis and that early treatment reduces mortality to a considerable extent (5, 15). The success of medical treatment is closely related to the height of the blood pressure at the outset of treatment (13). Furthermore the response to such treatment is equally good regardless of the cause of the hypertension. However important these items of information may be a proper evaluation of the patient with hypertensive disease still remains fundamental for arriving at the correct diagnosis and treatment. Current studies of etiology are necessary for disclosing basic knowledge of systemic arterial hypertension.

Over the past years such studies have mainly concentrated upon the frequency of renovascular hypertension providing figures from 1 to 20%. The extremes in this respect appeared recently in two simultaneous communications. In one of them 111 of 180 cases of hypertension were found to be caused by various types of renal arterial stenosis (10). The other failed to demonstrate any case of such a stenosis among 50 consecutive patients with unselected hypertension (1) despite of the fact that in 15 patients the disparity of the differential renal function test was typical of the presence of significant renal arterial obstruction (16).

In the present series of unselected hypertension the cause of the disease could be demonstrated in more than half of the patients. A significant difference between the sexes was apparent. Among men every second case was of the essential type

while this was so in only one third of the women. The reason seemed to be a preponderance in women of chronic pyelonephritis, Cushing's disease and allied disorders, collagen diseases and diabetes.

Quite another picture of the etiology was brought forward by a study of selected hypertension. Following a careful evaluation a total of 76 of the patients still maintained the diagnosis of essential hypertension and no significant sex difference could be observed. Moreover all the remaining cases (24%) proved to be hypertension of renal origin.

Separation of selected from unselected hypertension as made in this study thus provides results which are at great variance and thereby stress the heavy influence of selection. For comparative purposes such a procedure is an advantage. At present comparison between published series is more or less impossible due to ill defined terms of selection.

The selected hypertension results of the present series compare favorably with two recent Scandinavian series (6, 17). In all three studies the cause of the hypertension could be demonstrated in nearly 30% of the cases, the remaining 70% being cases of essential hypertension. However among the latter are many cases which cannot be regarded as adequately examined. The proportion fulfilling the more strict denomination pure essential hypertension consequently becomes much less. There is little doubt that the group of essential hypertension could be considerably reduced by complete examination of all the patients regardless of the severity of the disease. Renal angiography was for instance undertaken only in 45% of the patients of the Swedish series (6) in 33% of the series from Molde Hospital (17) and in 40% of the present series.

In other respects comparison between the above series is hardly possible due to various factors of selection. However it is worthwhile to consider the over representation of chronic pyelonephritis and aortic coarctation and the under representation of renal artery stenosis in the present series of unselected hypertension compared to the Swedish series (6).

The validity of methods is of prime importance for studies of etiology. In the present work urography with early sequential exposures was extremely helpful. Radioisotope renography added little information beyond that obtained by means

of urography. In fact renography led to a number of misleading observations. This is no wonder as nobody has yet proved what renography really reveals in respect of renal function (8).

Differential renal function tests were not consistently employed in this study because the reliability of these tests in predicting the presence of renal artery stenosis (16) cannot be trusted (1). In addition the ureteral catheterization represents a source of infection. Nor can the differential tests decide the causal relationship between the arterial stenosis and the coexisting hypertension (7, 10).

This is also true of renal angiography. However this method has the advantage of always providing reliable information on the patency of the renal arteries. Despite the fact that significant artery obstructions have been found in normotensive individuals (4) demonstration of such lesions remains a keystone in the evaluation of hypertension.

Renal biopsy is extremely helpful in arriving at the correct diagnosis (7). In reflecting the severity of the hypertensive disease and in predicting the result of surgery upon blood pressure, however the value of renal biopsy must be seriously questioned (10, 12).

Estimation of renin and angiotensin has hitherto been regarded as the only means by which the causal relationship between renal ischemia and hypertension could be established. However studies of human renal and essential hypertension have yielded conflicting results so far (11). Similarly the angiotensin infusion test has proved more or less useless in separating essential from renal hypertension (2).

Further progress in evaluation of hypertension clearly requires better methods than those available at present. This also applies to the diagnosis of primary aldosteronism which was not found in this series, a fact incompatible with the frequency of this condition according to Conn (3).

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IDIOPATHIC RETROPERITONEAL FIBROSIS

A Case of an Unusual Localization Effectively Treated with Glucocorticoid

Erik Juhl

From Surgical Department A Bispebjerg Hospital Copenhagen Denmark

Abstract A case of idiopathic retroperitoneal fibrosis of an unusual site at the upper pole of the right kidney is reported. The case was confirmed histologically and treated with glucocorticoid for nine months. This resulted in definite clinical improvement and complete normalization of the elevated ESR and abnormal serum electrophoresis. Compression of the inferior vena cava demonstrated by cavography was significantly less marked after the corticoid medication. The patient has been followed for eight months after discontinuation of the treatment and throughout this period he has remained symptom free and fully capable of working. As a conclusion from this case of idiopathic retroperitoneal fibrosis and from reports in the literature the author feels that all cases of idiopathic retroperitoneal fibrosis should be treated with glucocorticoid in high doses over a long period also when surgery is indicated.

Idiopathic retroperitoneal fibrosis (IRF) is a rare condition. It has been known since 1948 (14) but its aetiology is still partially unknown. Recently it has been related to the intake of methysergide maleate (Deserit*) (3).

The disease most often affects males between 40 and 60 years of age. The clinical picture is uncharacteristic consisting of fatigue, weight loss, gastrointestinal symptoms and low back pain. There may be fever, anaemia and an elevated ESR.

These symptoms and laboratory findings correspond to the active period of the disease when the morbid process is spreading in the retroperitoneal space. This is followed by a phase of healing which may give rise to ureteral compression and consequently signs of obstructive uropathy. Pyelography at this stage shows a characteristic appearance of medially displaced and narrowed ureters.

Pathologically the disease presents itself as a 2-5 cm thick fibrous infiltration localized on the

posterior abdominal wall on a level with the bifurcation of the aorta. The lesion extends laterally to the ureters which it envelops.

Proximally it seldom spreads farther than the lower poles of the kidneys and the distal limit is usually the promontory of the sacrum.

Histologically the appearances are predominated by firm collagen connective tissue with lymphocytes and plasma cells. In addition there may be polymorphonuclear leukocytes and fatty vacuoles. Thus the histological picture is non-specific.

Surgical treatment usually consists in freeing and peritonealization of the ureters. Nephrectomy or nephrectomy may be necessary in the more advanced stages. Previously reports have been published of X-ray irradiation (8, 12), antiphlogistics (6, 9), cytostatics (8) and antibiotics but without any convincing effect.

Treatment with glucocorticoid has also been reported by a few authors (1, 2, 10, 13, 15) apparently with a favourable effect but as other treatment has often been administered concurrently it is difficult to assess the results.

The present report concerns a case of an unusual site which was confirmed by biopsy. The only treatment given was long term glucocorticoid medication with well-substantiated success.

CASE REPORT

The patient was a 53-year-old man who was referred in March 1966 from a medical department for exploratory laparotomy because of a suspicion of pancreatic cancer.

The patient's father had died of gout. There was no family history of collagen diseases and the patient had not received Deserit.

In 1955 and 1965 he had undergone operations for bilateral inguinal hernia. During the latter stay in hospital

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unlike the previous films a normal axis for the right kidney and the previous blurring of the right psoas was no longer demonstrable

Follow up eight months after the glucocorticoid medication had been discontinued showed that the patient had maintained his body weight that he was still symptom free and fully capable of working. However the ESR and γ -globulin were somewhat elevated. The follow up is being continued in case a repeated course of prednisone should be needed.

DISCUSSION

The diagnosis in the present case is beyond doubt but the case differs from most of those reported previously in the unusual localization of the fibrosis around the upper pole of the right kidney. However there have been previous reports on abnormally situated IRF (4, 17) and co-existence of fibrosing mediastinitis and IRF has been described (5).

In this case the steroid therapy is of particular interest. There were unmistakable clinical improvement and definite objective criteria of a beneficial effect of the glucocorticoid medication. No other treatment was given simultaneously.

Steroid therapy has previously been used to a slight extent but as a rule concurrently with other treatment such as surgery (1, 5), radiotherapy (8), cytotoxics (8) or various antibiotics (17). Therefore it has been difficult to assess a possible beneficial effect. In a very few cases with a histologically confirmed diagnosis steroid therapy has been used alone (2, 10, 13, 15). In cases when the therapeutic result has been followed up there seems to have been a favourable effect of the steroid medication if it is given in high doses over a long period.

In our opinion the striking effect in the present case combined with the data given in the literature speaks for long term glucocorticoid therapy in high doses in all cases of IRF also those for which surgery is indicated.

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ACUTE HEMODYNAMIC CHANGES FOLLOWING BETA ADRENERGIC BLOCKADE IN HYPERTHYROIDISM

Ingemar Cullhed and Anders Parrow

From the Departments of Medicine and Clinical Physiology, University Hospital, Uppsala, Sweden

Abstract The acute hemodynamic effects of beta receptor blockade in thyrotoxicosis have been studied in six cases. The blockade was effectuated by the intravenous injection of 0.1 mg/kg/min of Inderal® (five cases) or Apun® (one case). In the four cases which at rest showed hyperkinetic circulation there was a substantial decrease in heart rate and cardiac output while the oxygen consumption was unaltered. On the basis of these hemodynamic findings which confirm earlier reports as well as some case reports on successful peroral therapy the use of these drugs in thyrotoxicosis is recommended. Their use is in most cases only temporary until full benefit is derived from measures directly aimed at the thyroid hyperfunction.

The hyperkinetic circulation in hyperthyroidism (26) closely resembles that seen in states of increased adrenergic activity. There is experimental and clinical (10, 13, 20) evidence of increased sensitivity to catecholamines in induced and spontaneous hyperthyroidism. Adrenaline has also been used as a diagnostic test in cases of suspected hyperthyroidism (13, 20). The exact mechanism seems however to be unknown and the question is still open whether there is an altered production, metabolism or organ sensitivity to catecholamines in hyperthyroidism.

The fundamental experimental work by Brewster et al. (4) demonstrated that the circulatory effects of induced hyperthyroidism could be abolished by total sympathetic blockade with local anesthetic drugs. They also reviewed its clinical use in some early reports dating back to 1936.

Sympathicolytic drugs have successfully been used in the treatment of thyrotoxicosis. The circulatory manifestations as well as the lid retraction have been successfully treated with reserpine (8) and guanethidine (12, 14, 27). The great value of Arfonad® in thyroid crisis has been reported from our clinic (19). It must be stressed that the meta-

bolic symptoms of thyroid hyperfunction are not influenced by these drugs though there might be some decrease in oxygen consumption and rate of weight loss.

During the last few years specific beta adrenergic blocking drugs have become available. From a pharmacological point of view these substances should be preferred to the sympathicolytic agents in the treatment of circulatory symptoms of thyrotoxicosis. Reserpine and guanethidine may cause diarrhea and water retention. Reserpine may cause psychic disturbances and guanethidine must be given in very large doses. The purpose of this work was to study the immediate effects of beta adrenergic blocking drugs on the central circulation in patients with Graves disease.

MATERIAL

There were five women and one man (Table I) all suffering from hyperthyroidism. The diagnosis was verified by careful clinical examination and standard laboratory procedures. Sinus rhythm was present in all cases. Weight loss was moderate in four, pronounced in one and absent in one case. All had a goiter as well as a vascular bruit. In case E.B. this murmur was unusually strong with a grade 3 (scale 1-6) continuous murmur audible over the goiter and irradiating down to the fourth intercostal space and high up in the neck. An arterio-venous fistula was suspected and a thoraco-cervical aortography performed. This revealed no shunt but a high contrast density in the thyroid gland.

The laboratory findings (Table I) reflect the varying degree of severity. Thyroidectomy was later successfully performed in four cases. The preoperative treatment with thyreostatic drugs was instituted after the examinations to be reported here. Thyroid crisis was not seen in this material.

METHODS

Right heart catheterization was performed with standard technique. Catheters were positioned in the brachial artery

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METHOD

Right heart catheterization was technique. Catheters were posi-

Table I *Clinical and laboratory findings*

Case	Age	Sex	Weight loss	Struma	Exopht.	Brunt	BMR ()	PBI (μ g/100 ml)	TIT uptake ()	I ¹²⁵ uptake (1 h μ)
S D	36	♀	+	+	0	(+)	+48	13.7	38.9	37.5
H F	52	♂	+	+	+	—	+46	15.3	—	30.4
M H	40	♀	++	+	+	+	+52	9.7	25.5	—
E. B	36	♀	0	+	+	+	+31	7.3	31.9	81.8
I T	56	♀	+	+	+	+	+54	14.0	37.8	23.8
B B	30	♀	+	+	0	+	+47	—	35.5	—

BMR = basal metabolic rate PBI = protein bound serum iodine TIT = triiodothyronine uptake in red blood cells I¹²⁵ = uptake in thyroid gland during the first hour
(+) = questionable + = moderate ++ = marked

and the pulmonary artery. After 10 min rest the cardiac output was determined according to Fick's principle. Oxygen consumption was measured during 10 min. In the middle of this period blood samples were drawn and pressures registered. The active drug was then administered, with 0.1 mg/kg body weight/min. In five cases propranolol (Inderal[®]) was injected in one case H 56/28 (dl 1-(*o*-allyl phenoxy)-3-isopropylamino-2-propanol hydrochloride Apun[®] (1)) in the same dose. The drugs were given via the catheter into the pulmonary artery. Ten min after the injection the cardiac output and the pressures were again measured.

In cases E. B. and B. B. studies were made at rest as well as during supine exercise on an electrically braked

bicycle ergometer. In case I. T. cardiac output was measured at rest, 10 min after a saline injection and then 10 min after injection of the active agent.

RESULTS

These are presented in Table II. Tachycardia was presented in only three cases. In four cases the arterio-venous oxygen difference (AVD) was low 28.4–34.5 ml/l with an increased cardiac index, 5.1–6.3 l/min/sqm. The two cases with a normal

Table II *Hemodynamic findings*

Case	Age	Period	Heart rate	V _{O₂} (ml/min)	AVD (ml/l)	CO (l/min)	CI (l/min/m ²)	SV (ml)	R _g (u)	R _p (u)
S D	36	Rest, before	116	276	31.3	8.8	6.3	76	—	1.2
		Rest, after 4 mg Inderal	83	266	39.6	6.7	4.8	81	—	1.4
H F	52	Rest, before	84	345	42.1	8.4	4.4	88	10	1.8
		Rest, after 7 mg Apun	81	326	43.9	7.4	4.0	92	11	—
M H	40	Rest, before	82	245	42.3	5.8	4.0	71	17	1.0
		Rest, after 5 mg Inderal	80	242	37.7	6.4	4.4	80	16	0.5
E. B	36	Rest, before	88	249	28.4	8.8	5.1	104	11	1.7
		Work, 300 kpm/min	128	891	56.6	15.7	9.1	122	8	1.4
		Rest, after 6 mg Inderal	65	216	41.0	5.3	3.1	81	16	3.6
I T	56	Rest, before	100	277	33.3	8.3	5.4	83	1	1.6
		Rest, saline	99	266	33.1	8.0	5.2	81	11	1.9
		Rest, after 5 mg Inderal	88	263	39.4	6.7	4.4	76	15	2.5
B B	30	Rest, before	116	275	34.5	8.0	5.8	69	12	1.1
		Work, 250 kpm/min	179	1122	93.8	12.0	8.7	67	11	1.8
		Rest, after 5 mg Inderal	101	286	43.1	6.6	4.8	66	14	—
		Work, 250 kpm/min	133	972	103.8	9.4	6.8	71	13	2.0

V_{O₂} = oxygen uptake. AVD = arterio-venous oxygen difference CO = cardiac output CI = cardiac index SV = stroke volume R_g = systemic arterial resistance R_p = pulmonary arterial resistance

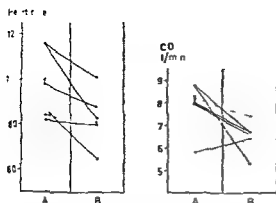


Fig 1 Heart rate and cardiac output at rest before (A) and after (B) beta adrenergic blockade. Straight lines represent cases with Inderal; interrupted line the case with Apun. The short vertical line in A represents the effect of placebo (saline) injection.

AVD had a cardiac index within the upper normal range (4.0-4.4 l/min/sqm).

In two cases cardiac output was determined during exercise. In case E, B there was an exaggerated increase in cardiac output in relation to the increase in oxygen consumption while the increase in cardiac output in case B, B followed the normal regression line.

After beta receptor blockade there was a decrease in heart rate and cardiac output in only those four cases who had a hyperkinetic circulation before (Table II, Fig 1). This was caused by an increase in AVD though in case E, B the oxygen consumption also fell. In this case there was a substantial fall in stroke volume. The two cases with

normal AVD and heart rate showed no significant changes in heart rate or cardiac index.

The injection of saline in case I, T which was performed in exactly the same way as the injection of the active drug caused no significant changes in any studied parameter.

In one case the effects of propranolol were studied at rest as well as during exercise (Table II, Fig 2). Before the injection of propranolol the heart rate rose to high values at exercise and the patient became nearly exhausted. After Inderal the heart rate rose much less on the same work load and the patient tolerated the work test better.

There were only small changes in vascular resistances (Table II). No side-effects were noted.

DISCUSSION

The sympathomimetic, inotropic and chronotropic actions on the heart are mediated via the beta adrenergic receptors. Several drugs with a more or less specific blocking effect on these receptors have been synthesized. In 1964 Wilson et al (28) reported their findings with nethalide in 11 cases with spontaneous hyperthyroidism. In a later study from the same group (29) the effects of 0.15 mg/kg Inderal on induced hyperthyroidism in healthy subjects were reported. Both drugs inhibited the effects of isoprenaline infusion but the basal heart rate and cardiac output was not influenced. They concluded that the circulatory effects of spontaneous hyperthyroidism and induced hypermetabolism were not mediated through stimulation of beta adrenergic receptors.

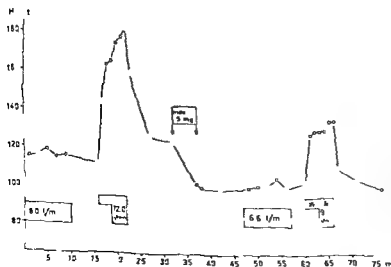


Fig 2 Heart rate in case B, B at rest and exercise (00 kpm/min) before and after the injection of Inderal. Rectangles with text Inderal and HOK indicate the time for injection and exercise respectively. Rectangles with values in l/min represent cardiac output and the time for its determination.

A favorable influence of Inderal[®] on the tachycardia of anxiety states has been reported (2, 15, 21, 24). In thyrotoxicosis a marked in some cases dramatic effect on heart rate was found when Inderal[®] was given in a single dose intravenously (5 mg) or by mouth (30 mg) (2, 24). The clinical effect of Inderal[®] in more prolonged peroral therapy in cases with severe thyrotoxicosis has been reported (5, 22). There was a very good effect on tachycardia, but also on other symptoms such as rapidity of weight loss, nervousness, tremor and lid retraction. As with reserpine and guanethidine the thyroid hyperfunction was not influenced. Recently two cases with thyroid crisis were advantageously treated with peroral Inderal[®] (6).

The hemodynamic response to a single intravenous injection of Inderal[®] was studied by Howitt and Rowlands (17). They injected 15 mg in 20 cases of Graves' disease. In all cases there was a significant decrease in heart rate at rest as well as during exercise while the cardiac output decreased in all but two cases. In our material the heart rate also decreased, though usually in two cases. We used a smaller dose which might not have resulted in an effective receptor blockade in all cases. However, no further changes were noted if 5 mg of Inderal[®] were added to a first dose of 10 mg (17). It has been found that Inderal[®] in a dose of 0.1 mg/kg causes a virtually complete beta receptor blockade in man (16). However, after 5–10 mg Inderal[®] intravenously isoprenaline will give a slight increase in heart rate but not in cardiac output (9).

The absence of response to Aptin[®] in one case could be due to the fact that this drug has a weak beta receptor stimulating action (1). However, the doses used (5 mg) Inderal[®] and Aptin[®] were found to be equipotential when the effect of isoprenaline infusion (0.09 µg/kg/min) was studied (18). Further, the case where Aptin[®] was given without demonstrable effect had a normal heart rate and cardiac output at rest. The same applies to the other case in which Inderal[®] was given without significant reaction. It must be admitted that a higher dose could have elicited a more pronounced effect. However, even with the relatively small doses employed in this study a clear effect was seen in four cases out of six, which were also those with the most pronounced hypermetabolism.

The effect of Inderal[®] on the circulatory re-

sponse to exercise is a decreased rise in heart rate and cardiac output compared with the changes at work before blockade (9, 11, 23, 25). These findings can be illustrated by Fig. 2. The lower values at rest after the first exercise period could be an effect of exercise itself and this applies also to the lower cardiac output during the second work test. However, these changes are probably more significant after work in sitting position than after supine work (7).

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Congress Announcements

The Second International Meeting of the International Society for Neurochemistry (ISN) will be held in Milan Italy September 1 to 5 1969

Secretaries

For details and registration forms contact Dr J Polch P1 Dept of Neurochemistry McLeans Hospital Harvard Medical School Belmont Mass 02178 USA or Dr Rodolfo Paoletti Institute of Pharmacology University of Milan Via Andrea del Sarto 21 20129 Milan Italy

The Fourth International Congress on Hyperbaric Medicine will be held at the Park Hotel Sapporo Japan September 2 to 4 1969

President Juro Wada MD

Secretary General Takashi Iwa MD

Secretaries Koji Ikeda MD and Dorothy J Boone Dept of Thoracic and Cardiovascular Surgery Sapporo Medical College South 1 West 16 Sapporo Japan

The Tenth International Cancer Congress with four preliminary special sessions will be held in Houston under the auspices of the International Union Against Cancer (UICC) May 22 to 29 1970

Information from National Organizing Committee Secretariat The University of Texas MD Anderson Hospital and Tumor Institute 6723 Bertner Avenue P O Box 20465 Astro dome Station Houston Texas 77025

Chairman R Lee Clark MD

Secretary General Murray M Copeland MD

International Congress of Radiology in the Netherlands

The European Association of Radiology will hold its 2nd Congress in the Netherlands Congress Centre at The Hague June 14 to 18 1971

The organization has been entrusted to the Netherlands Association of Radiology

President of the Congress Professor J R von Ronnen of Leiden University

Approximately 2000 participants are expected to attend Information from the local secretariat c/o Holland Organizing Centre 16 Lange Voorhout The Hague

THE PLASMA LIPIDS AND THEIR FATTY ACID PATTERN IN MYOCARDIAL INFARCTION

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Abstract The concentrations of total plasma lipid the major plasma lipid fractions including cholesterol, triglycerides and phospholipids and the fatty acid composition of the lipid fractions mentioned were determined partly by gas liquid chromatography in 21 male and 11 female patients suffering from acute myocardial infarction during the first 1-2 days of their disease and—in the survivors—4 to 17 months later. The results were compared with those of 18 male and 10 female normal persons without any known coronary disease and whose age was comparable with that of the patients.

In most cases the results for the acute patients did not differ significantly from those for the controls. The analyses of the re-examined patients showed increased concentrations of plasma total lipid and cholesterol as compared with those of the controls.

The fatty acid composition of the main plasma lipid fractions was not significantly changed in the two patient groups as compared with that of the controls with one exception: very low or zero values of linoleic acid of the plasma lipid fraction were significantly more frequent in the acute patients as compared with the controls.

The importance of lipids in the pathogenesis of atherosclerosis is still controversial. From some prospective studies it appears, however, that the risk of experiencing a clinical episode of coronary disease is amongst others a function of plasma cholesterol level (1, 17, 23).

With the advance of gas liquid chromatography much attention has been paid to determination of the fatty acid patterns of the different lipid fractions in human blood. Whereas this technique is too laborious and time consuming for prospective investigations, only a few studies have compared the composition of lipids in sera of normal persons and of patients with coronary arterial disease (4, 5, 9, 13, 15, 22, 28). By and large only few differences have been observed.

The present paper is a report on a study of the main plasma lipid fractions and their fatty

acid patterns from C14:0 through C20:4 in apparently healthy controls and in patients with acute myocardial infarction. As far as it was possible the patients were examined twice: in most cases initially within the first two days after the actual attack and subsequently between two and twelve months later. Thanks to a modification of the technique applied it has been possible to obtain a fairly accurate estimate of the fatty acids which are but scantily represented.

MATERIAL AND METHODS

The material comprises 21 male and 11 female patients, mean age 64 years (range 40-91 years) and 66 years (range 55-76 years) respectively. All the patients had unequivocal myocardial infarction according to the conventional criteria.

On the first morning after admission within 8-7 hours average 30 hours after onset of the acute attack, blood specimens were drawn from the fasting patient (patient group I). This examination was repeated for 13 of the male patients representing all the survivors between 4 and 12 months later on an average 6 months and for the 6 surviving female patients between and 13 months later average 8 months (patient group II).

The control material consisted of subjects without any known coronary or other atherosclerotic disease (controls). The material comprised 18 men, mean age 50 years (range 33 to 69 years) and 10 women, mean age 48 years (range 35 to 62 years). Blood specimens were drawn in a state of fasting.

The different analytical methods for lipid assay and their respective accuracy have been described previously (1). The gas liquid chromatographic recorder was provided with an automatic attenuator. This device allowed relatively large amounts of lipid material (0.05-0.1 microg) to be put on the column. Consequently the peaks of the minor fatty acid components could be better represented than is usually the case, whereas the peaks of the major components could be kept within suitable limits by automatically reducing the sensitivity of the recorder from one to several times by a factor two (Fig. 1).

The gas liquid chromatographic results were obtained

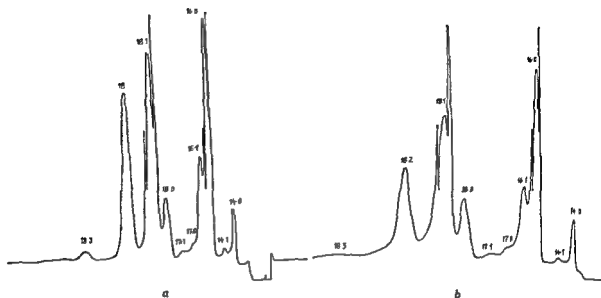


Fig 1 Gas liquid chromatogram of the fatty acids of plasma triglyceride from a control (a) and a patient (group I) (b) The difference in the concentration of linolenic acid is remarkable

as a percentage of the fatty acids present in the different plasma lipid fractions: cholesterol esters, triglycerides and phospholipids. The chromatographic analysis of the non-esterified fatty acid (NEFA) was omitted as this fraction is quantitatively insignificant.

RESULTS

The mean values \pm SD obtained for the major plasma lipid fractions in patient groups I and II are compared mutually and with those of the controls in Table I. Statistical analyses (Student's *t* test) were carried out to detect any significant differences between the three groups studied. Regarding the controls versus male patient group I

no such differences were found. In female patient group I the total lipids and the triglycerides were significantly higher than those of the controls. In comparison with the controls, both sexes in patient group II had significantly higher values for total lipids and total cholesterol. The same applied to cholesterol esters in the female patients. During the course of the disease—comparing patient group I with group II—total lipids, total cholesterol and phosphatides increased significantly in the male patients. The serum triglycerides decreased in both sexes but significantly so in the females only.

Table I Concentration of plasma total lipids, cholesterol, cholesterol esters, triglycerides and phosphatides in controls and in patient groups I and II

d = difference between mean values *signific* = statistical significance of difference *ns* = non significant *p* = probability

	Sex	Controls	Patient group I	Patient group II	Patient group I—controls		Patient group II—controls		Patient group I—patient group II	
					<i>d</i>	<i>Signific</i>	<i>d</i>	<i>Signific</i>	<i>d</i>	<i>Signific</i>
Total plasma lipids	♀	618 ± 86	774 ± 211	772 ± 124	156	-(<i>p</i> < 0.05)	154	+(<i>p</i> < 0.02)	2	<i>ns</i>
	♂	666 ± 111	678 ± 138	793 ± 116	12	<i>ns</i>	127	+(<i>p</i> < 0.001)	94	+(<i>p</i> < 0.05)
Plasma cholesterol	♀	209 ± 36	234 ± 57	276 ± 53	25	<i>ns</i>	67	+(<i>p</i> < 0.02)	42	<i>ns</i>
	♂	211 ± 29	215 ± 62	279 ± 36	4	<i>ns</i>	68	+(<i>p</i> < 0.001)	62	+(<i>p</i> < 0.001)
Plasma cholesterol esters	♀	249 ± 41	296 ± 112	333 ± 173	47	<i>ns</i>	87	+(<i>p</i> < 0.01)	40	<i>ns</i>
	♂	255 ± 133	272 ± 71	299 ± 56	17	<i>ns</i>	44	<i>ns</i>	27	<i>ns</i>
Plasma triglycerides	♀	78 ± 37	182 ± 111	102 ± 111	104	+(<i>p</i> < 0.01)	24	<i>ns</i>	80	+(<i>p</i> < 0.05)
	♂	144 ± 37	144 ± 56	123 ± 70	0	<i>ns</i>	21	<i>ns</i>	21	<i>ns</i>
Plasma phosphatides	♀	230 ± 23	258 ± 73	265 ± 43	28	<i>ns</i>	35	<i>ns</i>	7	<i>ns</i>
	♂	234 ± 40	225 ± 47	259 ± 38	9	<i>ns</i>	25	<i>ns</i>	34	+(<i>p</i> < 0.01)

Table II *Fatty acids of the plasma cholesterol esters as per cent*

Fatty acids	Sex	Controls	Patient group I	Patient group II	Patient group I —controls		Patient group II —controls		Patient group I— patient group II	
					d	Signific	d	Signific	d	Signific
C14 0	♀	0.5±0.31	0.2±0.2 ^a	0.3±0.37	0.3	n.s.	0.2	n.s.	0.1	n.s.
C16 0		12.2±2.72	10.7±2.01	13.5±3.30	1.5	n.s.	1.3	n.s.	2.8	n.s.
C16 1		4.2±1.48	4.7±1.61	6.3±0.81	0.5	n.s.	2.1	n.s.	1.6	n.s.
C18 0		0.7±0.22	0.6±0.24	0.5±0.71	0.1	n.s.	0.2	n.s.	0.1	n.s.
C18 1		18.9±2.64	22.9±3.28	22.5±3.51	4.0	n.s.	3.6	n.s.	0.4	n.s.
C18 2		51.0±4.68	49.2±8.80	46.6±3.33	1.8	n.s.	4.4	n.s.	2.6	n.s.
C18 3		0.4±0.38	0.2±0.35	0.3±0.3	0.2	n.s.	0.1	n.s.	0.1	n.s.
C20 4		4.8±0.58	5.3±1.69	4.3±1.95	0.5	n.s.	0.5	n.s.	1.0	n.s.
C14 0	♂	0.4±0.24	0.4±0.36	0.4±0.23	0	n.s.	0	n.s.	0	n.s.
C16 0		12.5±2.50	10.9±2.34	12.1±1.03	1.6	n.s.	0.4	n.s.	1.2	n.s.
C16 1		4.2±0.97	4.4±1.88	5.6±2.11	0.2	n.s.	1.4	n.s.	1.2	n.s.
C18 0		1.2±0.69	0.6±0.38	0.6±0.22	0.6	n.s.	0.6	n.s.	0	n.s.
C18 1		25.2±6.70	22.6±3.86	22.4±3.39	2.6	n.s.	0.8	n.s.	0.2	n.s.
C18 2		45.9±6.88	48.9±7.27	49.1±4.53	3.0	n.s.	3.2	n.s.	0.2	n.s.
C18 3		0.5±0.37	0.2±0.34	0.3±0.34	0.3	n.s.	0.2	n.s.	0.1	n.s.
C20 4		4.1±1.75	4.0±1.23	4.5±1.11	0.1	n.s.	0.4	n.s.	0.5	n.s.

Abbreviations: see Table I

The fatty acid patterns of the three major lipid fractions in the controls and in the two groups of patients are shown in Tables II-IV. The peaks recorded for C20 0, C20 1, C20 2 and C20 3 were in each case too low to permit calculation. The values for linolenic acid (C18 3) will be further dealt with separately in Table V. The values obtained \pm s.d. for the different fatty acids were subjected to statistical analyses to detect any significant differences between these values in the controls and in the patients (groups

I and II) suffering from coronary infarction. No such differences were observed.

The frequency of extremely low values obtained for linolenic acid (C18 3) is given in Table V. The zero values include extremely low values inaccessible for computation. In both groups of patients of both sexes the incidence of such low values was far higher than in the controls. However, this difference has been shown to be significant only for patients in group I (both sexes).

Table III *Fatty acids of the plasma triglycerides as per cent*

Fatty acids	Sex	Controls	Patient group I	Patient group II	Patient group I —controls		Patient group II —controls		Patient group I— patient group II	
					d	Signific	d	Signific	d	Signific
C14 0	♀	1.8±0.81	0.5±0.14	1.3±1.06	1.3	n.s.	0.5	n.s.	0.8	n.s.
C16 0		27.3±3.22	27.0±5.52	25.7±3.38	0.3	n.s.	1.6	n.s.	1.3	n.s.
C16 1		5.2±2.56	8.6±2.01	10.0±2.92	3.4	n.s.	4.8	n.s.	1.4	n.s.
C18 0		5.5±1.70	5.4±1.52	4.4±0.78	0.1	n.s.	1.1	n.s.	1.0	n.s.
C18 1		35.5±4.2	40.8±4.75	43.1±3.73	5.3	n.s.	7.6	n.s.	2.3	n.s.
C18 2		14.5±2.63	14.2±3.5	12.7±5.10	0.3	n.s.	1.8	n.s.	1.5	n.s.
C18 3		0.7±0.8	0.3±0.32	0.6±0.47	0.4	n.s.	0.1	n.s.	0.3	n.s.
C14 0	♂	1.4±0.59	0.7±0.56	0.2±1.06	0.7	n.s.	1	n.s.	0.5	n.s.
C16 0		9.4±2.74	27.5±4.58	30.1±4.25	19	n.s.	0.7	n.s.	2.6	n.s.
C16 1		5.7±1.46	6.9±2.15	8.6±1.53	1.2	n.s.	2.9	n.s.	1.7	n.s.
C18 0		6.1±2.75	5.0±1.6	4.9±1.12	1.1	n.s.	1.2	n.s.	0.1	n.s.
C18 1		42.4±6.4	43.1±4.07	39.4±3.0	0.7	n.s.	3.0	n.s.	3.7	n.s.
C18 2		11.3±2.53	13.0±3.09	13.2±3.79	1.7	n.s.	1.9	n.s.	0.2	n.s.
C18 3		0.9±0.33	0.5±0.37	0.8±0.37	0.4	n.s.	0.1	n.s.	0.3	n.s.

Abbreviations: see Table I

Table IV *Fatty acids of the plasma phospholipids as per cent*

Fatty acids	Sex	Controls	Patient group I	Patient group II	Patient group I—controls		Patient group II—controls		Patient group I—patient group II	
					d	Signific	d	Signific	d	Signific.
C14 0	♀	0.2±0.11	0	0	0.2	n.s.	0.2	n.s.	0	
C16 0		30.0±1.87	29.0±1.05	33.4±2.71	1.0	n.s.	3.4	n.s.	4.4	n.s.
C16 1			1.3±0.53							
C18 0		16.4±1.45	15.1±2.69	17.0±1.70	1.3	n.s.	0.6	n.s.	1.9	n.s.
C18 1		13.9±2.42	18.6±2.05	15.8±2.51	4.7	n.s.	1.9	n.s.	2.8	n.s.
C18 2		22.5±1.91	19.8±2.63	20.7±3.09	2.7	n.s.	1.8	n.s.	0.9	n.s.
C18 3		0.4±0.11	0.3±0.30	0.3±0.29	0.1	n.s.	0.1	n.s.	0	n.s.
C20 4		8.6±1.35	8.6±1.74	7.0±1.55	0	n.s.	1.6	n.s.	1.6	n.s.
C14 0	♂	0.2±0.13	0.1±0.15	0.1±0.21	0.1	n.s.	0.1	n.s.	0	
C16 0		30.7±1.06	33.1±6.08	32.3±4.30	2.4	n.s.	1.6	n.s.	0.8	n.s.
C16 1		1.2±0.57								
C18 0		17.5±3.63	15.3±2.93	16.5±1.26	2.2	n.s.	1.0	n.s.	1.4	n.s.
C18 1		16.1±2.69	15.9±2.38	16.0±2.78	0.2	n.s.	0.1	n.s.	0.1	n.s.
C18 2		19.9±4.38	20.8±5.11	24.6±3.38	0.9	n.s.	2.7	n.s.	1.6	n.s.
C18 3		0.5±0.28	0.3±0.35	0.5±0.41	0.2	n.s.	0	n.s.	0.2	n.s.
C20 4		7.6±2.07	7.5±2.03	7.4±1.86	0.1	n.s.	0.2	n.s.	0.1	n.s.

Abbreviations see Table I

DISCUSSION

As regards the major plasma lipid fractions the mean values recorded for the male controls are in fair accordance with those observed in larger materials from the more prosperous Western countries (2, 14, 22). As far as we are aware extensive studies of healthy females are lacking. In the present female controls however the plasma concentration of triglycerides is on an average remarkably low as compared with that of the males whereas there is a close agreement with respect to the concentrations of cholesterol and phospholipids in the two sexes.

Admittedly the controls were all apparently

healthy but being middle aged only few of them—if any—were free from atherosclerosis. It would thus be fallacious to consider the controls biochemically normal or even optimal (24, 27).

A study of Table I will show that in the male patients with recent coronary infarction (group I) the major lipid fractions are practically identical with those of the controls. On the other hand the corresponding female patients have significantly higher values for triglycerides than their controls. On re-examination (patient group II) both sexes were very much alike. Their total cholesterol was now significantly increased in comparison with the controls and the original remarkable elevation of triglycerides was largely eliminated in the females.

It has been shown repeatedly that acute myocardial infarction is followed by marked changes in the plasma lipids. This applies to cholesterol (3, 7, 16), plasma lipoproteins (7, 16) and to all the major plasma lipid fractions (26). The latter investigation is especially pertinent to the present study. According to this the changes in cholesterol and phospholipids were uniform. Both decreased significantly during the first week after infarction but regained the initial level after a further two weeks. Subsequently no substantial changes were recorded. The triglycerides rose markedly from the second day, culminating after three weeks. Here the initial level was not reached.

Table V *Frequency (in per cent) of values of α -linolenic acid in the main plasma lipid fractions in controls and patient groups I and II*

	Sex	Cholesterol esters (of zero)	Triglycerides (of zero)	Phospholipids (of zero)
Controls	♂	36.4	0	0
Patient group I		80.0	40.0	40.0
Patient group II		50.0	33.3	42.8
Controls	♀	2	0	10.0
Patient group I		60.0	35.0	50.0
Patient group II		38.5	7.7	23.1

until after about twelve months. The present "normal" cholesterol values reflect in all probability the usual initial decrease in this lipid. The rather high cholesterol values recorded on re-examination are more likely to be representative of the pre infarction level. This assumption must however be based on experiences from other studies (27).

Some evidence emphasizes the importance of triglycerides in the genesis of myocardial infarction (6, 8, 18, 19). Whereas the findings for the male patients in the present investigation do not lend support to this hypothesis, the observations in the females are at first sight more suggestive. They involve the low triglyceride values in the female controls as compared with those of the males, and the high values recorded shortly after the acute attack. The latter phenomenon may however simply be an expression of the above mentioned rise in this lipid immediately following myocardial infarction. On re-examination triglyceride values in female patients and controls do not differ significantly.

Concerning the fatty acid composition of the major lipid fractions, a study of Tables II and IV corroborates the well known considerable difference in the fatty acid pattern of the triglycerides, cholesterol esters and phospholipids. As regards this pattern there are no significant differences between the two sexes. Furthermore it is notable that the fatty acid pattern of the two groups of patients closely resembles that of the controls. Several authors have reported that the serum cholesterol esters of subjects with atherosclerosis contain a lower percentage of linoleic acid (C18:2) than that of healthy subjects (4, 5, 9, 15, 22, 28). The present findings are however in accordance with the observations of Lawrie et al. (13) who did not observe any differences in the cholesterol ester fatty acid composition between healthy subjects and patients with ischaemic heart disease. As regards the fatty acid patterns of triglycerides and phospholipids it is generally accepted that they are not influenced by atherosclerotic disease (13, 22, 25).

In 1964 Owren et al. (20) stated that the polyunsaturated fatty acid linolenic acid (C18:3) should be regarded as having an almost specific significance for thrombocyte adhesiveness. Increased platelet adhesiveness which is considered of importance for a tendency to thrombosis and

which is regularly found in atherosclerosis, diabetes and other conditions should be reduced by adding linseed oil or linolenic acid to the food. In 1965 however Owren et al. (21) admitted that the described effect of linolenic acid in decreasing platelet adhesiveness could not be reproduced in patients with coronary heart disease or other atherosclerotic diseases.

After the publication of Owren's first paper it was considered worthwhile to subject the minor fatty acids to a more detailed examination than hitherto. The result of this investigation has been the observation of a remarkably high frequency of very low or zero concentrations of the linolenic acid in the three major plasma lipid fractions presented in Table IV. In relation to coronary heart disease it is so far not possible however to attribute any pathogenetic significance to linolenic acid or rather to its absence.

ACKNOWLEDGEMENT

Aided by a grant from Statens Almindelige Videnskabsfond.

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ORAL TREATMENT OF PERNICIOUS ANEMIA WITH HIGH DOSES OF VITAMIN B₁₂ WITHOUT INTRINSIC FACTOR

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Abstract The concluded results of an experimental study are reported confirming that the absorption of vitamin B₁₂ takes place in two ways 1 through the mediation of intrinsic factor 2 without mediation of intrinsic factor

The former mechanism allows a B₁₂ absorption in normally IF sensitive cases of pernicious anemia and in normal persons with an upper limit of about 2 µg. The latter mechanism allows an absorption of vitamin B₁₂ which is roughly proportional to the oral dose administered and amounts to about 12% of the dose within a very wide dose range. Thus it is possible to increase the uptake to any desired level. This direct uptake was found to be of the same magnitude irrespective of whether the patient had a normal absorption was suffering from pernicious anemia with or without resistance to intrinsic factor preparations or had some other form of disturbed B₁₂ absorption (idiopathic malabsorption postoperative state after extensive gastric or intestinal resections or ileitis). The result of a long term clinical trial is also reported in which a daily oral dose of 500-1000 µg of vitamin B₁₂ without intrinsic factor was given to patients with pernicious anemia and other types of vitamin B₁₂ deficiency. The material comprised 64 patients followed for up to more than 5 years. After the remission period very few serum B₁₂ determinations showed borderline or subnormal values and low levels were never of long duration. At the conclusion of the study the serum B₁₂ values were well within the normal range in all cases. Also the individual mean values of the B₁₂ determinations made during the observation period from the second month of treatment were normal in all cases. No neurological complications were observed. The blood values were normal in all but a few cases in which concurrent diseases were present (malignancy chronic infections iron deficiency states). Experimental evidence obtained indicates that oral treatment with 500 µg of B₁₂ daily has also resulted in replenishment of the B₁₂ deposits. The treatment with high oral doses of vitamin B₁₂ thus constitutes a convenient and completely reliable maintenance therapy in pernicious anemia and other states of B₁₂ deficiency and is a fully acceptable alternative to the conventional method of vitamin B₁₂ injections. This type of therapy (1000 µg B₁₂ daily) was introduced into Sweden in 1964 and is now extensively used.

During recent years many reports have been published suggesting that high doses of vitamin B₁₂ alone administered orally can produce a satisfactory effect on pernicious anemia (3 4 6 8 9 10 11 12 15 16 17 18 19 23 24 25 26 27). However other investigators have not studied quantitatively how pernicious anemia patients absorb vitamin B₁₂ from the intestine without the mediation of intrinsic factor (IF) nor have they studied the absorption process in cases of pernicious anemia with an acquired resistance to IF preparations or in other types of B₁₂ deficiency. Furthermore most earlier clinical studies have been made with lower B₁₂ doses which cannot be expected to give a safe long term effect in all cases. In the present report an account is given of the quantitative experimental studies presented in part in earlier papers (3 4) which confirmed that absorption of vitamin B₁₂ takes place in two ways.

1 Absorption through the mediation of IF

Quantitative absorption studies employing the radioactive isotope technique have shown that the absorption of a certain quantity of B₁₂ administered together with an IF preparation increases when the IF dose is increased but only up to a certain maximum (1 4). When the dose of B₁₂ given in combination with an optimum amount of IF varied within the range of 1-100 µg normally IF-sensitive patients with pernicious anemia absorb on an average a maximum of 0.5-2.2 µg of the given dose or about the same quantity as the uptake in healthy persons producing their own IF (3 4 and present). Patients with partial resistance to IF absorb less of the B₁₂ dose but

cases be induced to absorb the same quantity of B_{12} if the IF dose is greatly increased (1)

2 Absorption without mediation of IF

The studies presented in this paper which were briefly reported earlier demonstrate that the amount of vitamin B_{12} absorbed from the intestine without the mediation of IF corresponds to approx 1.2% of the dose administered within a very wide dose range. (In a previous report (4) the uptake was calculated to be approx 1.5%. The new average figure 1.2% is derived from a more comprehensive material and based on statistical analysis.)

Thus by increasing the B_{12} dose sufficiently it is possible to increase the uptake to any desired level since a fairly constant ratio between dose and uptake has been demonstrated throughout the entire dose range from 1 μg to 100 000 μg (100 mg!). The mechanism is not known but the demonstrated relationship between dose and uptake indicates the possibility of a passive diffusion process for which we have used the term direct uptake to differentiate from uptake mediated by IF.

It is particularly noteworthy that the direct uptake was found on an average to be of the same magnitude irrespective of whether the patient had completely normal absorption, was suffering from anemia with or without resistance to iron preparations or had some other form of disturbed absorption due to idiopathic malabsorption, ileitis, total gastrectomy, ileocectomy or jejunectomy.

Finally a summary is given of long term clinical trials in pernicious anemia up to more than 5 years in which oral doses of 500–1000 μg vitamin B_{12} without IF were administered daily. A preliminary report has been presented earlier (4). The results have shown that the treatment is definitely safe and provides a fully acceptable alternative to injection therapy (Behepan tablets 1 mg B_{12}). It is effective both in pernicious anemia and in other forms of B_{12} malabsorption. Thus older preparations containing IF together with vitamin B_{12} which are less reliable are in our opinion no longer justified (4, 5).

MATERIAL AND METHODS

In the experimental studies the oral B_{12} doses were tagged with ^{57}Co or ^{59}Co (Metek & Co). In most experi-

ments where a very small fraction of the dose was expected to be absorbed 0.75 μC was given as $B_{12}\text{-Co}^{57}$. Otherwise a radioactivity dose of 0.1–0.5 μC was administered.

The IF concentrate used was a dry commercial preparation from hog pylorus mucosa with a B_{12} -binding capacity of approx 0.2 μg of vitamin B_{12} per mg (2). Of this material 50 mg exert an optimal IF effect with 1 μg of vitamin B_{12} in ordinary pernicious anemia. The IF dose needed for maximal absorption increases up to about 500 mg when the B_{12} dose is increased to 10 μg or more (3).

The dose of tagged B_{12} was mixed with the IF concentrate (if any) in water prior to administration. The doses were given on an empty stomach if not otherwise specified.

The meal used in some tests was standardized to contain bread, butter, cheese, milk and coffee. In these tests the dose was given one half hour after the meal.

An intramuscular flush dose of 1000 μg of non-radioactive cyanocobalamin was given 2 hours after the dose.

After each dose 0–24 h urine collections were made. Samples of the urines were evaporated to one tenth of their original volume. The radioactivity of 5 ml portions of the concentrates was measured with a Tracerlab detector in an arrangement for spectrometric analysis of Co^{57} or Co^{59} peaks.

The uptake of vitamin B_{12} was calculated according to Callender and Evans (7) from the 24 hour urinary radioactivity excretion after conversion of radioactivity to μg of vitamin B_{12} and multiplication by 3. The value thus obtained is not exact since it is based on measurement of excretion not absorption. However as discussed in this and earlier reports (3, 4) this method gives an approximate value of the amount of B_{12} absorbed which is sufficiently reliable for the validity of the practical conclusions drawn.

The diagnoses of the different B_{12} deficiency states included in the clinical study were established in each case by the use of a battery of examinations including ordinary hematological tests, bone marrow aspiration, determination of serum B_{12} , analysis of gastric juice, Schilling tests (with few exceptions) and other tolerance and balance tests of gastrointestinal function. The serum B_{12} determinations were made with the Euglena gracilis technique by Dr A. Hallander, Institute of Medical Chemistry, University of Uppsala, Sweden.

EXPERIMENTAL STUDIES

Before clinical studies were started extensive experiments were carried out to study the absorption of radioactive vitamin B_{12} given orally with and without IF at varying doses and in various clinical conditions.

Vitamin B_{12} without IF in pernicious anemia

Tests were carried out with doses varying from 1 μg to 100 000 μg . Fig. 1 shows the calculated uptake in a double logarithmic diagram. For each dose level mean values of calculated uptake and standard deviation are plotted and the number of patients tested is specified.

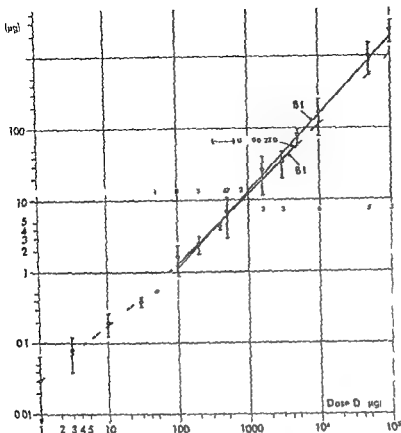


Fig 1 Calculated uptake of vitamin B_{12} given orally without intrinsic factor to pernicious anemia patients. Mean standard deviation and number of subjects are specified for every dose level. BI is calculated regression line for interval 100–100 000 μg . BII is calculated regression line for interval 100–5000 μg coincides closely with $U = 0.01 \times D$ i.e. uptake = 1.2% of dose.

BI is regression line for the interval 100–100 000 μg .
BII for the limited interval 100–5000 μg .
BI was calculated separately for two reasons:

1. this range has a greater interest for clinical practice
2. at doses exceeding 5000 μg a significant urinary excretion is obtained also without flush, for this and other reasons (see below) the uptake as calculated may be slightly too high in this upper region.

Equation for regression line BI (100–100 000 μg)

$$U = 0.00798 \cdot D^{0.97}$$

Equation for regression line BII (100–5000 μg)

$$U = 0.0146 \cdot D^{0.97}$$

It is interesting to note that the equation for BI within its interval coincides very closely with the simple equation $U = 0.01\% \cdot D$ i.e. the calculated uptake equals 1.2% of the dose.

Consequently the average uptake of vitamin B_{12} given orally without IF in pernicious anemia is about 1.2% of the dose within a very wide dose range.

The regression lines do not comprise the range below 100 μg since at low doses the average relative uptake is higher as evident from Fig 1.

The natural explanation is the presence of some IF activity remaining in some patients which will influence the total average uptake at low doses.

Vitamin B_{12} without IF in subjects with normal absorption

Tests were carried out with doses from 1 to 800 μg . Fig 2 curve BIII shows the calculated uptake. Mean values, standard deviations and number of subjects tested are specified as in Fig 1.

The curve has been drawn by manual fitting. No regression line has been calculated since it cannot be expected to be a straight line and the material is not comprehensive enough to warrant, with any certainty, the calculation of a curve with an equation of a higher degree.

Regression line BI (see Fig 1) is included for comparison.

The result shows that the average absorption at doses from 1 to 100 μg increases slowly from approx. 0.5 to 2 μg . As a consequence of the presence of IF in the subjects' gastric juice, this absorption is far above the absorption found in pernicious anemia (Fig 1). At higher dosage the absorption curve rises more rapidly as the direct

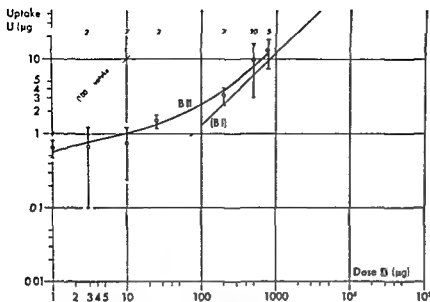


Fig 2 Calculated uptake of vitamin B_{12} given orally without intrinsic factor to test subjects with normal B_{12} absorption. Mean standard deviation and number of subjects are specified for every dose level. Curve BII is drawn by manual fitting. Regression line BI (Fig 1) is included for comparison.

proportional uptake dominates over the IF mediated absorption. The difference between healthy subjects and pernicious anemia patients becomes smaller as the dose is increased showing that the IF mediated absorption is limited and of little importance for the total uptake at high B_{12} doses. The B_{12} uptake in normal subjects has also been studied by Gaffney et al (12) Heinrich et al

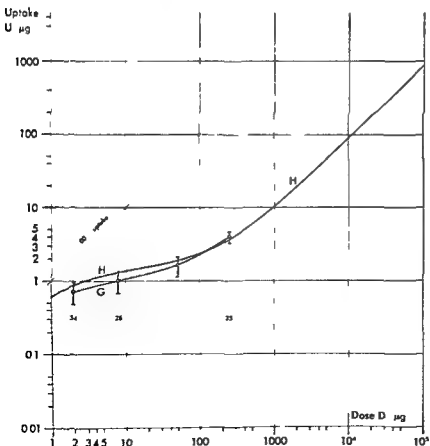


Fig 3 Calculated uptake of vitamin B_{12} given orally without intrinsic factor in normal subjects, according to other investigators. G Uptake calculated from urinary excretion values found by Gaffney et al (12). H Curve according to an equation for the relationship between dose and uptake suggested by Heinrich et al (15).

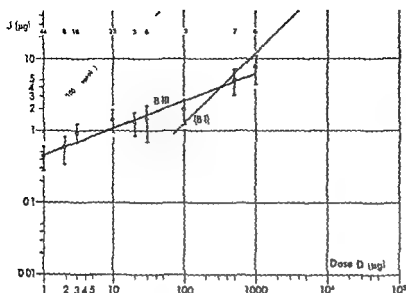


Fig. 4. Calculated uptake of vitamin B_{12} given orally with an adequate dose of hog intrinsic factor to pernicious anemia patients. Mean standard deviation and number of subjects are specified for every dose level. B_{III} is calculated regression line. Regression line B_I (Fig. 1) is included for comparison.

(15) and Heinrich and Wolfstetter (16). Their results are presented in Fig. 3, curves G and H respectively.

The average absorption values and standard deviations plotted for curve G were calculated from urinary radioactivity excretion values presented by Gaffney et al. in the same way as the uptake was calculated in our own studies.

Curve H represents an equation suggested by Heinrich et al. for the relationship between dose and total uptake in healthy subjects:

$$U = \frac{15D}{D+15} + \left(1 - \frac{15}{D+15}\right) 0.009D$$

This equation was based on values obtained from total body measurements of absorbed radioactivity after oral administration of radioactive B_{12} .

It is evident that curves G and H differ very little and they are also in good agreement with our curve B_{II} .

Since the B_{12} absorption ought to be about the same in pernicious anemia patients as in healthy subjects when the doses are high (mainly direct absorption independent of IF), one might also expect good agreement between the upper part of curve H and our curve B_I . Above a dose of 1000 µg Heinrich found in healthy subjects 0.9–1% absorption (total body measurement) while we found in pernicious anemia approx. 1.2% absorption between 100 and 5000 µg (calculated from urinary excretion). The agreement is fairly good considering the very different techniques used.

Vitamin B_{12} with IF in pernicious anemia

Tests were carried out with doses from 1 to 1000 µg vitamin B_{12} together with an optimal amount of IF concentrate. The subjects were ordinary pernicious anemia patients, i.e. were known not to have developed refractoriness to hog IF. From earlier studies (1) we know that IF refractory patients absorb much less B_{12} in this test unless the IF dose is increased very considerably.

Fig. 4 shows the calculated uptake. Mean values, standard deviations, and number of subjects tested are specified as before.

Curve B_{III} is the regression line calculated from the values obtained.

Equation for regression line B_{III}

$$U = 0.455 D^{0.7}$$

Theoretically the curve cannot be a straight line in this type of diagram since it would cross the 100% absorption limit if extended downwards. Within the interval, however, it ought to give an acceptable description of the relationship between dose and uptake.

As expected, the uptake is within a wide range approximately the same as that obtained by vitamin B_{12} alone in normal subjects (curve B_{II}). At high B_{12} doses and correspondingly high IF doses, however, the uptake becomes lower than that obtained from B_{12} without IF in pernicious anemia patients or in normal subjects. Obviously the direct B_{12} uptake is hampered by IF and other B_{12} binding material in the hog IF concentrate. At B_{12} doses from a few hundred µg and up, the IF dose was far higher than the amount normally present in the gastric juice of a healthy subject.

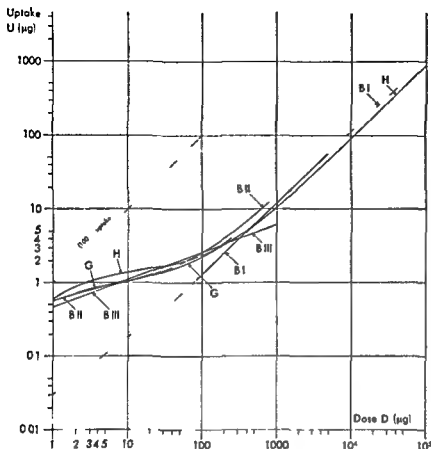


Fig 5 Calculated uptake of vitamin B_{12} curves presented in Figs 1-4 combined for direct comparison B I without intrinsic factor in pernicious anemia B I Berlin et al., regression line for interval 100-5000 μg B without intrinsic factor to normal subjects B II Berlin et al curve by manual fitting G Gaffney et al (17) curve by manual fitting H Heinrich et al (15) according to equation B_{12} with hog intrinsic factor in pernicious anemia B III Berlin et al regression line for interval 1-1000 μg

In Fig 5 our curves BI BII and BIII are presented in one diagram together with curve G and curve H from Fig 3 Here a direct comparison of the uptake under various conditions can be made and also a comparison of our results with those of other investigators

Vitamin B_{12} without IF in various conditions individual variations

In addition to the experiments reported above a series of tests has also been made in totally gastrectomized patients and in cases of other types of B malabsorption

Table I shows the calculated uptake in different conditions at the 500 μg dose the level for which the largest number of determinations is available

It is evident that the uptake of a high dose of vitamin B_{12} alone is largely the same regardless of the condition of the subject However the average uptake in pernicious anemia patients tends to be slightly lower than in normals probably as a consequence of the lack of IF in their gastric juice A small difference of this magnitude is to

be expected At this dose level the IF mediated portion of the total uptake is low compared to the direct uptake (cf curves BI and BII) The direct uptake in pernicious anemia may also be expected to be about the same whether the patients have developed refractoriness to hog IF or not

Fig 6 illustrates the variation at the 500 μg dose level in the entire material Only 3 out of the 74 subjects showed an uptake below 3 μg (the lowest value is 1.8 μg) and 8 had values exceeding 15 μg or 3% of the dose The majority (70%) showed an uptake within the interval 4-10 μg or 0.8-2% of the dose

Effect of flush dose at high B_{12} doses

If the oral B_{12} dose is very high the uptake approaches or exceeds 1000 μg i.e. the size of the intramuscular flush dose For this reason a significant excretion can be expected from high oral B_{12} doses also without flush

A series of tests was carried out with and without flush when B_{12} doses of 500 μg and more

Table II Effect of intramuscular flush dose on urinary B_{12} excretion after high oral doses of vitamin B_{12}

Oral dose of radioactive B_{12} (μ g)	Per cent of radioactivity recovered in urine			
	With flush		Without flush	
	0-24 h	0-48 h	0-24 h	0-48 h
500	0.55	0.65	0.03	0.05
500	0.48	0.57	0.01	0.02
500	0.43	0.52	0.02	—
500	0.60	0.75	0.09	0.10
500	0.92	1.18	0.05	0.11
500	0.30	0.42	0.00	0.02
500	0.89	1.49	0.01	0.02
500	0.70	1.18	0.05	0.10
5 000	0.55	—	0.03	0.05
5 000	0.71	1.10	0.24	0.27
5 000	0.43	0.54	0.06	0.19
10 000	0.67	0.85	0.12	0.25
10 000	0.79	0.98	0.69	0.80
10 000	0.97	1.08	0.51	0.54
25 000	0.28	0.57	0.18	0.19
50 000	0.35	0.6	0.94	1.08
50 000	0.69	0.91	0.75	0.83
50 000	0.65	1.00	0.57	0.67
50 000	—	—	0.53	0.77
100 000	0.48	0.70	0.41	0.58
100 000	0.97	1.16	0.88	1.09

* The intramuscular flush dose of 1000 μ g of non radioactive B_{12} was given 2 h after the oral dose and repeated 24 h after the oral dose

It is obvious that the uptake is moderately higher when the dose is taken on an empty stomach. The dose should therefore preferably be taken before or between meals although this does not seem to be a critical point.

CLINICAL STUDIES

Several authors have attempted to determine the daily requirement of vitamin B_{12} in order to obtain a criterion for establishing the necessary daily uptake in the treatment of pernicious anemia.

Table III Calculated uptake (μ g) of vitamin B_{12} on an empty stomach and after a standard breakfast

Oral dose 500 μ g radioactive B_{12} . Mean values and range for ten patients with pernicious anemia

	Fasting	After meal
Calculated uptake (μ g)	7.8 (4.8-13.4)	4.8 (1.8-7.5)

Table IV Observation period for long term oral treatment of pernicious anemia with 500-1000 μ g of vitamin B_{12} (64 cases)

All six patients treated for three years or less and a few of the others, died during the observation period for reasons not connected with the disease studied

Time (mo)	No of cases	Time (mo)	No of cases
70	2	35-39	3
65-69	4	30-34	—
60-64	11	25-29	1
55-59	17	20-24	1
50-54	8	15-19	—
45-49	5	10-14	1
40-44	11		

It may be assumed that about 2 μ g constitutes the daily turnover rate and an amount sufficient to prevent relapse in pernicious anemia (14, 20, 21). Even though other studies (13, 20, 22) have indicated a higher requirement a quantity of about 2 μ g corresponds fairly well to practical experience.

As demonstrated above an oral dose of 500-1000 μ g of vitamin B_{12} gives on an average an absorption of 6-12 μ g. Even when individual variations are taken into account a daily dose of this magnitude can be expected to give a safe maintenance effect.

Altogether the clinical material comprises 64 established B_{12} deficiency cases from three Swedish medical centers (Eskilstuna, Falköping and Linköping) all examined and supervised personally by one of the authors (R. Berlin or G. Brante). The patients were started on 500 or 1000 μ g of B_{12} daily during the last three years of the study. 1000 μ g daily were given throughout. Sixty-one of the patients have been under treatment more than 36 months, 44 more than 48 months and 17 during 60 months or more. A more detailed review of the observation period of the entire material is shown in Table IV. The clinical diagnoses are given in Table V.

The clinical results which are presented in Table VI (Fig. 7 and Fig. 8) are more positive throughout to our knowledge than any results reported earlier in oral treatment of pernicious anemia. It should be noted that in this study a strict evaluation of the effect was made including regular determination of serum B_{12} for all patients. The patients were encouraged to adhere strictly to

V Case material in long term clinical trials with 500 µg or 1000 µg of vitamin B₁₂ administered daily in tablet form

	Pernicious anemia		Malabsorption		Total gas trectomy	Total
	Ordinary	Resistant ^d	After ileal resections	Cause not established		
no of cases	18	37	4	4	1	64

Demonstrated resistance of various degrees to preparations containing hog intrinsic factor plus vitamin B₁₂ or liver extract falling values for Hb and red blood cells and/or low values for B₁₂ in blood serum during treatment with hog IF subnormal response in the Schilling test with B₁₂ + hog intrinsic factor

Table VI Results of oral long term therapy with 00-1000 µg of vitamin B₁₂ daily in tablet form

including four cases of pernicious anemia and other types of vitamin B₁₂ deficiency

Hb and RBC	Satisfactory values	Subnormal values	Total (4 cases)
	55 cases	9 cases ^d	
B ₁₂	Normal 64 cases	Subnormal 0 cases	

One gastric carcinoma one chronic pyelonephritis postoperative abscess of left thigh one metastasizing urinary bladder carcinoma one chronic uremia one gastric carcinoma menorrhagia + iron deficiency anemia one ileitis with ulcers one iron deficiency after total gastrectomy one idiopathic malabsorption

the medication and were interviewed at each visit for checking of their medication habits They were also instructed to discontinue the treatment for three days before the blood sample was to be taken in order to avoid temporary peaks in the serum B₁₂ level

The therapy was discontinued for six patients in order to study the time needed for reappearance of low serum B₁₂ values The patients were kept under strict clinical control Table VII shows that the serum B₁₂ values stayed well within normal limits in all the patients for about 4 months Three patients reached the lower normal limit of 150 pg/ml after about 5 months and one patient after 7

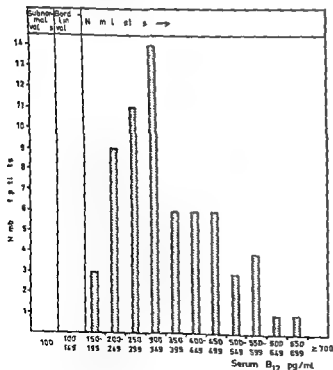


Fig 7 Results of oral long term therapy with 500-1000 µg vitamin B₁₂ daily in tablet form mean values of all serum B₁₂ determinations made from the second month of treatment Sixty four cases of pernicious anemia and other types of vitamin B deficiency

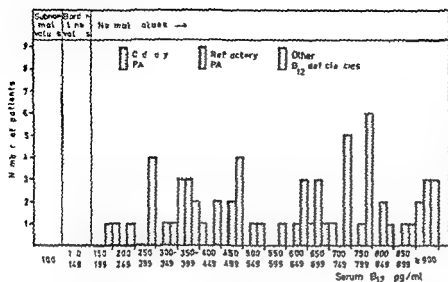


Fig 8 Results of oral long term therapy with 500–1000 µg vitamin B₁₂ daily in tablet form serum B₁₂ values at the end of the follow up period. The material (64 cases) is divided into groups according to type of B₁₂ deficiency

months. Two patients still had values within the normal range after 8½ and 7 months respectively. In a further case (B₁₂ deficiency after total gastrectomy) all B₁₂ therapy was interrupted by inadvertence after 44 months of oral treatment with 500 µg of B₁₂ daily. After 1½ years his serum B₁₂ value had decreased from 570 to 350 pg/ml. In no case did the values for hemoglobin, red cells, serum iron, iron binding capacity, serum lactic acid dehydrogenase or serum glutamic acid oxalacetic acid transaminase show any notable changes during the observation periods. The results indicate that oral treatment with 500 µg of B₁₂ daily had also replenished the B₁₂ deposits.

Fig 7 shows the mean values of all serum B₁₂ determinations made for each case after the initial two months of therapy. Fig 8 presents the last serum B₁₂ values determined in the follow up

study. In this figure the material is divided according to type of B₁₂ deficiency. Of all serum B₁₂ determinations made after 2 months of treatment or more comprising 562 values only 40 values were below 150 pg/ml and 6 values below 100 pg/ml. Generally, these low values were obtained during the early months of treatment of patients in definite relapse. Repeated low values on several consecutive occasions were found only in exceptional cases where 500 µg daily was given; they could easily be corrected by increasing the dose to 1000 µg daily. Thus the long term treatment with high doses of vitamin B₁₂ alone has resulted in serum B₁₂ levels well within the normal range in all cases.

It should also be noted that no negative reactions were recorded that could be attributed to the medication. No observations were made of

Table VII Serum B₁₂ values after discontinuance of oral B₁₂ therapy 500 µg daily (after the time for last value reported the treatment was resumed)

Patient and type of B ₁₂ malabsorption	Duration of treatment when discontinued (mo)	Serum B ₁₂ (pg/ml) time after discontinuance							
		Days		Months					
		3	10	1	3	4	5	6	7
1 Ordinary PA ^a	19	440	00	705	190	190	150		130
2 Ileal resection	3	515	460	210	210		110	140	
3 IF refractory	33	315	470	170	210		145	170	
4 Idiopathic malabsorption	33	390	400	55	290		110		135
5 Ileal resection	33	765	300	0		230		35	
6 IF refractory PA	33	570	510	735	265		260		20

^a PA = pernicious anemia

neurological disturbances indicating development of subacute combined degeneration of the cord. Absent ankle tendon reflexes recorded in a few cases were connected with concomitant diseases such as diabetes advanced arteriosclerosis or a history of scurvy. Absence of vibratory sensibility in the feet was reported in one case: an old woman with senile dementia.

Many patients spontaneously expressed satisfaction with the discontinuance of injections. At a special interview some patients declared that they did not care whether they received injections or tablets. No patients had any objection to oral treatment.

Since the uptake is proportional to the dose it has been possible to supply the entire daily requirement by one small tablet daily. Only very few patients tended to neglect the medication. In all but one a mentally deficient patient negligence could be corrected by information.

With regard to the daily standard dose—500 μ g or 1000 μ g—the higher dose was chosen. Although it was found both theoretically and through experience that 500 μ g per day gave satisfactory results the higher dose offers better protection in the event of temporary negligence in medication. Even at the higher dose the treatment is simple and inexpensive and no report has been received of any side reactions or other unfavourable effects from long term therapy.

The investigation has also demonstrated that it is possible to give oral treatment with high doses of vitamin B₁₂ not only as maintenance therapy but also as initial treatment during relapse without any untoward effects. For investigational reasons the maintenance dose has been used also initially throughout this study. In routine use we recommend however initial oral treatment of 2000 μ g twice daily during the first month or injection therapy in order to fill the body stores as rapidly as possible.

Our clinical experience has provided complete confirmation of the experimental results. It is now definitely established that the treatment of pernicious anemia with high oral doses of vitamin B₁₂ without IF is a fully satisfactory alternative to injection therapy not only in cases of ordinary pernicious anemia but also in those showing IF refractoriness and in other cases of B₁₂ malabsorption.

This treatment although the cost of the tablets

is somewhat higher than the cost of injectable B₁₂ is economically quite reasonable because it saves personnel and allows less frequent visits to the doctor. The tablets were introduced in Sweden in 1964 and are now in widespread use. No negative experiences have been reported in connection with their practical routine use.

ADDENDUM

In a review of his experimental work on oral B₁₂ without IF Heinrich (Ergebn. inn. Med. Kinderheilk. 25:1 1967) discusses the suitable daily maintenance dose and finds 300 μ g daily to be adequate.

Statistically this is correct because this dose results in an absorbed and retained average quantity of approx. 3 μ g or slightly more than the normal daily turnover. However, considering the variations in absorption, a wider safety margin seems to be appropriate in order to provide a safe standard therapy. Heinrich's figures as well as ours show some absorption values far below average and in our experience low values are found rather constantly in certain individuals. Heinrich refers to clinical trials published by others indicating that 300 μ g daily might be sufficient. Our more comprehensive material followed up for a much longer period of time showed that 500 μ g daily although in general adequate may give borderline serum B₁₂ values in a few cases. In these patients normal values were rapidly restored by 1000 μ g daily.

Considering that some patients may have a higher B₁₂ requirement or a lower uptake than average and further that (a) the absorption is slightly lower if the dose is taken with a meal (b) a reasonable allowance for occasional negligence should be taken into account in this type of self-medication (c) a dose of 1000 μ g daily has not caused any untoward reactions during 5 years' study and (d) the difference has little economic significance we have preferred to recommend 1000 μ g as daily maintenance dose.

Heinrich also discusses much higher oral doses at correspondingly longer intervals. We have seriously considered such alternatives since it is no doubt possible to work out other dosage regimens which are equally effective. In our opinion however it is necessary that an important self-medication like this can be included in a simple daily routine. The possible cost reduction gained by higher doses at longer intervals would most probably not be large enough to justify the increased risk of the patient's forgetting his tablet and the more serious consequences if a dose is neglected.

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TREATMENT OF ANGINA PECTORIS WITH BETA RECEPTOR BLOCKADE MODE OF ACTION

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Abstract In a double blind cross-over study the effect of the dextro isomer and the racemate of a beta receptor blocking agent (H56/78 \pm) was compared with that of a placebo in 13 patients with angina pectoris. The dextro isomer had no effect on attack rate. The inhibitive effect of racemate on angina pectoris was highly significant statistically. The decrease in the number of angina pectoris attacks was of such magnitude that it was considered also of clinical significance in the majority of the patients. The difference in effect of the dextro isomer and of the racemate indicates that the inhibitive effect on angina pectoris is due to the laevo isomer. The main beta receptor blocking activity resides in the laevo isomer and thus the beneficial effect on angina pectoris seems to be due to beta receptor blocking properties. No definitely serious side-effects were observed.

Several investigations published during the past two years seem to indicate that beta receptor blocking agents have a positive effect on angina pectoris as recently reviewed by Epstein and Braumwald (4). Such investigations are complicated by many factors even in controlled double blind trials as recently discussed (2). The so-called run in effect can interfere with a subsequent double blind trial by causing a continuing decrease of angina pectoris attacks during this trial (5) or by diminishing the symptoms to such an extent that the following double blind trial becomes difficult to evaluate (2). Such a run in effect on angina pectoris has been demonstrated also with agents that are ineffective (3). It is also important to control other factors such as a possible carry-over effect between trial periods as discussed by Rabkin et al (6). Moreover it seems necessary to perform double blind trials with the patients knowing that a placebo will be introduced during unknown periods of the trial otherwise

there appears to be a risk of a biased recording of attacks as pointed out previously (2).

Not until these technical problems are controlled is it possible to approach the more central question of the mode of action of beta receptor blockade in angina pectoris. It is thus not known whether the effect is actually due to beta receptor blockade or to other causes e.g. local anesthetic effects. This question has arisen because it seems that much higher doses are required in order to produce an effect on angina pectoris than those causing beta receptor blockade. Information on this matter seems to be of interest not only from the therapeutic point of view but also because it may contribute to our knowledge of the nature of angina pectoris. When planning the present study the difficulties referred to were avoided by selecting only patients with frequent attacks of angina pectoris. Furthermore the run in effect on the double blind trial was minimized by prolonging it to over four months and by administering the same number and size of tablets during the trial and not as in a previous study (2) increasing the number of tablets. Carry over effects were compensated for by analysing only late parts of the periods of the double blind trial. Finally patients were informed about the introduction of a placebo during unknown periods of the trial. The beta receptor blocking agent used was 1 (2-allyl-phenoxyl)-3-isopropylamino propanol (2)-hydrochloride (H 56/28 Aptin[®] AB Hassle Göteborg).

With this technique it was possible to investigate the effectiveness on angina pectoris of the dextro- and laevo isomers of H 56/28 by testing first the pure dextro-form and then for the same case material the racemate. This was of interest

Table 1 Clinical data and results

Pat no	Age	Sex	Diagnosis	Dextro trial		Racemate trial		Sub- jective impression ^b	BP ^c (mm Hg)
				No of attacks per 14 d		No of attacks per 14 d			
				Placebo	Dextro	Placebo ^a	Racemate		
1	69	♂	Ang. pect. hypertension	5	8	9 (8)	4	+2	00
2	76	♀	Ang. pect. diabetes mellitus hypertension	10	7	12 (8)	4	+4	-107
3	54	♀	Ang. pect. diabetes mellitus, hypertension cholelithiasis, hypercholesterolemia	7	14	8 (8)	7	-1	-25 -3
4	66	♂	Ang. pect.	11	5	2 (8)	0	+3	-2 10
5	63	♂	Ang. pect. hypertension	18	11	10 (4)	0	+5	00
6	66	♀	Ang. pect. hypercholesterolemia	9	9	10 (9)	7	+1	-10 -10
7	61	♂	Ang. pect. hypercholesterolemia	25	18	23 (30)	14	+1	-3 10
8	69	♀	Ang. pect.	6	10	6 (5)	3	+3	-157
9	70	♂	Ang. pect. hypercholesterolemia	111	106	84 (90)	55	+3	10 5
10	61	♂	Ang. pect.	4	10	6 (12)	4	0	0 0
11	51	♂	Ang. pect. hypercholesterolemia	52	42	3 (4)	17	0	0 0
12	66	♀	Ang. pect.	26	36	—	—	—	—
13	64	♂	Ang. pect. hypercholesterolemia hypertension	5	5	—	—	—	—
Sum				289	290	173 (186)	115		

^a Figures within parentheses refer to the placebo period immediately before the racemate trial (cf. Material and methods).

^b General condition during treatment (racemate) and placebo periods as estimated by the patient compared with the preceding weeks: unchanged 0 improved +1 much improved +2 worse -1 much worse -2. The table shows the score for the treatment period minus that for the placebo period.

^c The figures indicate change in systolic diastolic blood pressure calculated as mean systolic diastolic blood pressure during placebo periods minus that during treatment (racemate) period.

for the elucidation of the mode of action since isomers have approximately the same local anesthetic effect but the beta receptor blocking effect is much stronger in the laevo-form (1).

MATERIAL AND METHODS

Fifteen outpatients with angina pectoris were selected for the trial. Two of these had to be excluded early in the investigation because of inability to cooperate. The remaining 13 patients consisted of five women aged 54-76 and eight men aged 51-70. On exertion, all suffered from typically localized anginal chest pains which immediately subsided when patient was at rest. Nitroglycerin alleviated the pain. Seven patients had previously suffered from myocardial infarction. In none of these cases did infarction occur less than three months before the trial. Two patients were subjected to coronary angiography showing pronounced pathological lesions. When the study was begun all the patients had had not less than four (11 more than 7) attacks of angina pectoris per week, lasting at least two minutes. Six patients were treated with digitalis, with or without saluretics for previous congestive heart failure four for hypertension, and five for hyperlipemic conditions. Two had diabetes mellitus treated with diet and sulphonylurea. None had a history of obstructive lung disease or a tendency to mental

depression. During the trial no changes were made in previous medication including the above mentioned drugs and also long acting nitrite preparations (9 patients) sedatives and antispasmodic therapy. All took nitroglycerin but were recommended not to use it prophylactically.

That part of the study in which the dextro form of 11 56 28 was tested was started in March 1967 with a one week control period followed by a two-week placebo period. Then 50 75 and 100 mg of the dextro isomer were given four times daily: each dose for one week. Thereafter followed two double blind periods of placebo or 100 mg of the dextro isomer four times daily in randomized order each for three weeks. During this whole period of 13 weeks except for the first control week, four tablets of identical appearance and taste were given daily.

During weekly checkups patients' data-sheets were collected. History of the preceding week recorded and physical examination and ECG and other laboratory tests performed as described previously () with the exception that blood lipids and chest X-ray were not followed in all patients.

Immediately after the last double-blind period of the dextro trial a four week placebo period was introduced. This was during the vacation period (July). Frequency of attacks was recorded during the last two of these four weeks. Then followed two double-blind periods of placebo or 100 mg of the racemate of 11 56 73 g/m four times daily in randomized order each for three weeks.

During this part of the trial patients no 11 and 13 (cf Table I) were unable to take part because of lack of time. During this whole period of ten weeks four tablets of identical appearance and taste were again taken daily which means that during 16 weeks prior to the racemate trial the same number of similar tablets was taken daily.

During this latter part of the trial the number of attacks was recorded and the history and physical and laboratory status controlled as in the dextro trial.

Statistical calculations were made one tailed according to Wilcoxon rank sum test (7).

RESULTS

The results of the dextro trial are shown in Fig 1 and Table I. The number of angina pectoris attacks were practically identical when the last two weeks of the placebo and the treatment periods are compared. This applied also to nitroglycerin consumption (right half of Fig 1 left part of Table I).

This result seemed to justify regarding the whole of this part of the investigation as a run in period for the racemate trial. Therefore in the left half of Fig. 1 the number of attacks and the nitroglycerin consumption per week have been marked off with the weeks in consecutive order irrespective of whether placebo or the dextro isomer was given. It is then immediately apparent from the figure that the run in effect was very small.

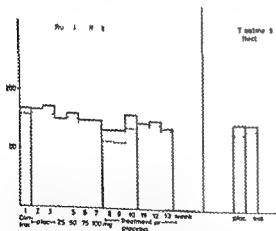


Fig 1 Number of attacks of angina pectoris and number of nitroglycerin tablets taken during different periods of the trial of the dextro isomer of H 56/28. Left part of figure shows effect of time on number of attacks and nitroglycerin consumption; right part shows average number of attacks and nitroglycerin tablets taken per week during the two last weeks of the double blind trial with the placebo or the drug, each for a total period of three weeks.

Furthermore the average number of angina pectoris attacks during weeks 11, 12 and 13 (see Fig 1) was not lower than that during weeks 8, 9 and 10 (144 and 142 respectively) showing that any possible run in effect had passed by this time.

The results of the racemate trial are given in the right part of Table I. All the patients had a smaller number of angina pectoris attacks when taking racemate as compared with the placebo results except for patient no 11. This difference is highly significant statistically ($p=0.006$). The nitroglycerin consumption was closely related with the number of attacks and is not presented. Eight out of the 11 patients remaining in this part of the trial had a positive score when they assessed their own condition while two rated themselves as 0 and one had a negative score. Whether the changes in blood pressure are of significance seems uncertain.

Side effects

The following observations may possibly be pertinent to a discussion of side-effects although they may equally well be unrelated to drug administration. For patient no 6 the serum glutaminoxalacetate acid transaminase values were 35 and 45 units for the last two weeks before the introduction of racemate 60 units during its introduction and 11 units immediately after. Initially patient no 3 had normal alkaline phosphatase values which rose during placebo administration to 17 and 14 units when on racemate her value was 12. This patient had cholelithiasis. Her other liver tests were normal. Patient no 5 developed gynaecomastia bilaterally during the trial. No changes in this condition were observed whether the patient received placebo tablets or the drug. No other subjective or objective signs of any possible side-effects were observed.

DISCUSSION

The first part of the study showed that compared with the placebo the dextro isomer of H 56/28 had no effect on the number of angina pectoris attacks. This result made it possible to consider the whole first part of this investigation as a run in period for the latter part where the racemate of H 56/28 was tested. It was also demonstrated that the attack rate was constant indicating that the run in effect of the utilized procedures had passed. This fact facilitates the interpretation of the results of the racemate trial which followed.

There was however a decrease of approximately 100 attacks in the placebo period immediately before the racemate trial (173 (186) see

sum of attacks at bottom of Table I) as compared with preceding periods (289-290). It should be noted that in addition to the missing attacks of the two patients who did not participate in this part of the trial this was almost entirely due to patient no 11 who spontaneously stated that his angina pectoris was much improved by his vacation. Consequently it is most probable that this decrease in the total number of attacks is not caused by a continuing run-in effect.

In a previous study (2) it was found that the number of angina pectoris attacks diminished markedly when the dose of the racemate was increased during the run-in period. In view of the information on the true magnitude of the run-in effect in the present work it seems probable that this marked decrease was caused to a significant degree by the drug even though the mode of tablet administration was different in the two investigations. The fact that in the study mentioned (2) frequency of attack did not again increase during the subsequent placebo periods to approximately the level of the control values might well be due to either a carry-over effect and/or the physical training of the patients (cf. 8) which was made possible during the run-in period because of fewer angina pectoris attacks.

When run-in effects and carry-over effects were excluded and a sufficient number of attacks remained to allow comparisons between periods the effect of the racemate in preventing angina pectoris attacks was found to be highly significant statistically.

The clinical significance of the effect of the drug evidently varied in different patients. Most of the patients were helped by the treatment. This is clearly seen in the number of attacks in patients no. 2, 5, 7 and 9. The condition of other patients also improved even though this is not so evident in the number of attacks. Patients no. 1, 4 and 8 were able to increase their physical activity and did so up to the limit where angina pectoris again occurred. This is most dramatically exemplified by patient no. 1 who when the trial started was unable to leave his house because of angina pectoris but when on racemate he climbed the roof of his house in order to repair it and while doing this again had angina pectoris. The rest of the patients however did not experience for certain any beneficial effect of the treatment (no. 3, 6, 10 and 11). Patient no. 11

even had an increased number of attacks of angina pectoris when treated with racemate. According to the patient this was probably due however to personal problems which caused anxiety and sleeplessness suddenly arising during the period of the racemate treatment.

It then seems possible to conclude that the racemate of H 56/28 has a definite statistical effect on the frequency of angina pectoris attacks. The clinical significance of this decrease in the number of attacks varies in different patients but no doubt most patients are helped by this treatment. What characterizes these patients who experience a beneficial effect is not apparent at present. The dextro isomer of H 56/28 has only a local anesthetic effect and very weak beta-receptor blocking activity (1). It is not effective in the treatment of angina pectoris. Therefore the local anesthetic effect of the racemate probably does not account for the prevention of angina pectoris attacks. It appears probable that this effect is exerted by the beta-receptor blocking activity of the laevo isomer of the racemate.

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FREE FATTY ACIDS GLYCEROL AND ALVEOLAR ACETONE IN OBESE WOMEN DURING PHENFORMIN TREATMENT

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Abstract One patient, acting as her own control was studied for twenty months. She lost weight when on phenformin and gained weight when this was discontinued. A group of moderately obese subjects lost 13 kg in four weeks on phenformin as against 0.8 kg on placebo. In a second group with resistant obesity no weight changes were observed. The alveolar concentration of acetone and the plasma concentration of FFA did not change significantly during the observation periods whereas the plasma concentration of glycerol was significantly reduced during the phenformin period.

Concomitant with the increasing prevalence of administering phenformin hydrochloride to patients with diabetes it has been observed that phenformin has effects over and above those on carbohydrate metabolism. During recent years this drug has been particularly recommended to obese diabetics. This indicates that phenformin could be of special importance for fat metabolism (1, 3, 7, 14). Very few studies of phenformin have been made on non-diabetic patients (12). Pedersen (11) treated four non-diabetic obese subjects with metformin which is closely related to phenformin and has a similar action. He observed that during caloric restriction the weight loss was greater with metformin than without it.

It was incidentally observed that a non-diabetic patient on caloric restriction lost weight considerably faster on phenformin. In a subsequent preliminary trial three out of five women showed a marked weight loss on phenformin whereas other forms of treatment had failed to produce this result. It was therefore of interest to ascertain whether the weight reducing effect of phenformin could be observed in a control clinical study. In view of the interaction between the carbohydrate metabolism and the fat metabolism free fatty acids (FFA) and glycerol in plasma were deter-

mined and the acetone in the alveolar air was estimated as an indication of the levels of the arterial ketone bodies (13).

MATERIAL

One overweight woman (weight 9 kg, height 166 cm) a nurse born 1918 without any family history of diabetes and normal glucose tolerance test was studied for twenty months with regard to weight loss on a caloric intake of 1500 kcal/day with and without phenformin administration and for one period on a caloric intake of 500 kcal/day. She became obese during the last ten years and was meticulously attentive to caloric intake during the study.

Two series of subjects were studied in a controlled clinical trial. The first consisted of 13 female subjects who considered themselves overweight and wanted to reduce. According to Natvig's standard four of them were not overweight. The patients were observed every fortnight when they were weighed and their alveolar acetone concentration determined. The fasting, plasma concentrations of FFA and glycerol were determined on venous blood after both a fortnight on phenformin and a fortnight on placebo. Each subject was one month on placebo and one month on phenformin. The phenformin dose was 50 mg in enterocoated capsules twice a day. The drugs were allocated according to the usual double blind technique.

The second series consisted of 18 women and three men with long-standing obesity which had been treated for a considerable time at the Department of Medicine. At the time that the study was in progress they were all living at home and attending the outpatient department. Earlier they had taken part in a control clinical study to test an appetite depressant. These patients were regarded as representing a particularly therapy resistant group. They were seen once a month.

Weight and concentration of acetone in the alveolar air were followed as in the previous group. In this series the plasma concentrations of FFA and glycerol were determined after one month either on placebo or on phenformin. Not all of the subjects were fasting, as some had taken coffee or tea and one or two pieces of bread and butter about two hours before the venous blood samples were drawn.

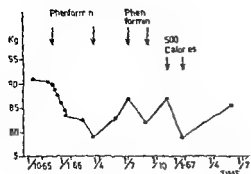


Fig 1 The weight curve of the nurse born in 1918

The patients were only given the drugs either phenformin or placebo no dietary instructions were given for either series

METHODS

The acetone concentration in the alveolar air was determined by gas-chromatography according to Rooth and Olsson (13)

Plasma FFA were analyzed by a colorimetric method described by Laurell and Tibbling (9) and plasma glycerol by an enzymatic fluorometric micro-method according to Laurell and Tibbling (8)

RESULTS

The weight changes in the nurse studied for twenty months are shown in Fig 1. During the first twelve months she was on the same caloric intake. When taking phenformin she rapidly lost weight whereas when the drug was not adminis-

tered she gained weight. However she twice ceased taking phenformin because of persistent headache. The mean weight loss during the two periods on phenformin was 2.7 kg/month and 2.9 kg/month respectively. On 500 kcal/day she lost weight faster than when on phenformin (6.0 kg/month) but was distressed by painful sensations of hunger which forced her to stop banting.

The weight and the weight changes, the concentrations of FFA and glycerol in plasma, and the acetone concentration in the alveolar air are given in Table I for the first series and in Table II for the second series.

It will be seen that in the first series the mean weight reduction after four weeks on placebo was 0.8 kg and on phenformin 1.3 kg. The mean individual weight reduction on phenformin was 0.5 kg more than that on placebo. This difference was not statistically significant ($t = 1.42$, $n = 13$, $0.1 > p > 0.05$). In the second series there were no differences in the weight changes at all either on placebo or phenformin. In both series the mean glycerol concentration was lower after phenformin than after placebo. Although the glycerol concentrations were higher in the second series the mean values of the individual differences were similar in both series and the results have therefore been pooled. The mean reduction after phenformin administration was 0.022 m moles/l ($t = 2.67$, $n = 33$, $p < 0.01$).

Table I Series no 1

Case no	Age (y)	Weight (kg)	Weight changes kg		Glycerol m moles/l		Free fatty acids m moles/l		Acetone μ g/l alveolar air	
			After 4 weeks on Phenformin	Placebo	After 2 weeks on Phenformin	Placebo	After 2 weeks on Phenformin	Placebo	After 2 weeks on Phenformin	Placebo
1	52	66.0	-0.5	0.5	0.055	0.075	0.26	0.30	1.14	0.40
2	52	71.4	-1.9	+1.0	0.106	0.064	0.72	0.52	0.12	0.68
3	47	72.7	0.9	-1.0	0.080	0.051	0.58	0.30	0.90	0.57
4	53	69.0	0	0	0.103	0.136	0.44	0.45	0.90	1.50
5	23	92.2	-2.2	-1.0	0.122	0.194	0.60	0.65	0.45	—
6	51	87.1	1.9	-3.1	0.059	0.10	0.47	0.39	1.00	1.37
7	18	88	-1.6	-1.7	0.051	0.101	0.66	0.81	0.80	0.80
8	24	81.0	-1.3	-0.4	0.051	0.062	0.49	0.48	0.53	0.33
9	52	62.5	-1.3	-0.6	0.089	0.140	0.69	0.68	0.84	1.05
10	38	66.7	-1.2	+0.5	0.058	0.056	0.42	0.37	0.28	—
11	49	105.4	-1.0	-1.1	0.070	0.084	0.61	0.34	0.35	1.20
12	53	58.3	-0.9	-0.3	0.167	0.212	0.84	1.09	0.67	1.71
13	45	85.7	-2.2	-2.4	0.079	0.086	0.71	0.63	2.55	1.73
Mean	42.9	75.2	-1.3	-0.8	0.084	0.106	0.58	0.54	0.96	1.05
S.E. of mean \pm	3.6	3.93	0.18	0.36	0.010	0.015	0.043	0.064	0.196	0.164
S.D. \pm	12.8	14.2	0.63	1.25	0.034	0.052	0.16	0.23	0.71	0.54

Table 11. Series no. 2

Case no.	Age (y)	Weight changes kg			Free fatty acids mmoles/l			Acetone μ g/l al. solar air		
		Initially weight kg	After 4 weeks on		Initially	After 4 weeks on		Initially	After 4 weeks on	
			Phenformin	Placebo		Phenformin	Placebo		Phenformin	Placebo
Fasting										
1	50	104.3	-0.7	+1.2	0.117	0.089	0.004	2.8	0.68	0.32
2	55	145	+3.6	+4.0	0	0.240	0.348	---	0.72	1.10
3	45	81.6	-2.6	+0.9	0.074	0.056	0.095	0.86	0.48	0.43
4	39	133.7	-2.4	-2.5	0.132	0.164	0.185	2.50	1.38	1.28
5	49	88.1	+1.6	-1.6	0.111	0.039	0.053	0.75	0.54	1.11
6	56	138.4	-1.3	-2.1	---	0.294	0.205	3.30	1.90	1.04
7	63	89.1	-4.3	-0.6	0.088	0.074	0.077	---	0.70	2.23
8	49	116.4	+2.4	0	0.198	0.197	0.104	0.54	0.64	1.08
9	42	121.0	+1.4	-2.1	---	0.025	0.074	---	1.30	0.76
10	37	8.5	+0.2	+1.2	---	0.064	0.074	0.43	1.18	0.21
11	19	90.3	-0.3	+0.2	0.063	0.039	0.085	1.10	0.34	2.10
12	33	90.7	-0.7	+0.2	---	0.147	0.064	---	0.72	0.47
13	64	113.8	-1.1	+7.3	0.156	0.12	0.059	0.18	0.29	0.39
14	43	117.8	+0.7	+0.9	0.087	0.065	0.107	1.51	0.54	0.90
Mean	49	107.5	-0.46	+0.14	0.172	0.120	0.138	1.14	0.94	0.94
S.E. of mean \pm	3.17	5.57	0.46	0.51	0.018	0.021	0.07	0.70	0.143	0.180
S.D. \pm	11.86	20.89	2.10	1.81	0.053	0.079	0.096	0.885	0.533	0.672
Non fasting										
15	52	91.6	0.8	-0.8	0.150	0.170	0.180	1.35	1.20	1.51
16	64	84.9	0	0.2	0.136	0.090	0.141	0.31	0.61	0.31
17	51	90.0	0	0	0.142	0.073	0.134	1.00	0.21	0.50
18	47	87.4	0.8	1.0	0.145	0.128	---	0.92	1.08	1.30
19	6	71.6	+1.1	0.9	0.084	0.081	0.103	0.59	0.68	0.52
20	55	100.8	1.6	1.9	0.064	0.134	0.091	0.43	2.15	0.38
21	50	81.0	1.1	1.3	0.077	0.089	0.064	0.6	0.64	0.23
Mean	49	86.7	0.03	0.11	0.114	0.174	0.136	0.98	0.94	0.71
S.E. of mean \pm	4.69	3.44	0.63	0.38	0.014	0.072	0.021	0.22	0.35	0.189
S.D. \pm	17.9	8.41	1.67	1.01	0.037	0.058	0.052	0.586	0.676	0.500
Total										
Mean	49	100.6	0.17	1.05	0.118	0.12	0.117	1.19	0.94	0.86
S.E. of mean \pm	5.5	4.9	0.42	0.35	0.011	0.014	0.018	0.189	0.10	0.135
S.D. \pm	11.56	19.7	1.88	1.54	0.047	0.069	0.082	0.760	0.535	0.602

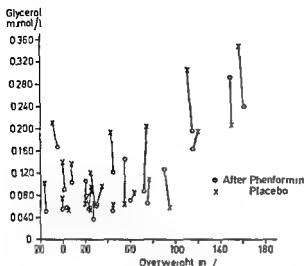


Fig 2 The correlation between the plasma glycerol concentration and the relative overweight in both series

When comparing the placebo period with the phenformin period there were no consistent changes in either FFA or the acetone concentration

As there did not appear to be a linear correlation between glycerol and overweight (Fig 2) or FFA and overweight, statistical significance was tested by rank correlation. In the first series there were no significant correlations between the concentration of glycerol and overweight or FFA and overweight whereas in the second series there were. Here as previously mentioned over

weight was considerable. When the study was begun the coefficient of correlation was significant at the 5% level correlating glycerol to overweight and FFA to overweight. When the patients were given phenformin and placebo the corresponding values were $r=0.72$, $n=16$, $p<0.001$ and $r=0.61$, $n=16$, $p<0.01$ respectively. FFA versus overweight was significant at the 1% level in both periods.

There was a linear correlation between FFA and glycerol as shown in Fig 3. The coefficient of correlation for the whole material i.e. in both series on placebo and on phenformin was 0.74, $n=67$, $p<0.001$.

DISCUSSION

Phenformin alone has no influence on the weight of subjects with resistant obesity as evidenced by the second series. These patients had previously been given various forms of treatment and there was no general weight reducing effect due to the fact that they were checked by a physician and their weight regularly controlled etc. In the first series, consisting mainly of moderately obese women, the placebo effect resulted in a mean weight reduction of 0.8 kg in one month. The additional weight loss when on phenformin was not statistically significant, although the mean weight reduction was 1.3 kg. However, if this observation is viewed in conjunction with the well

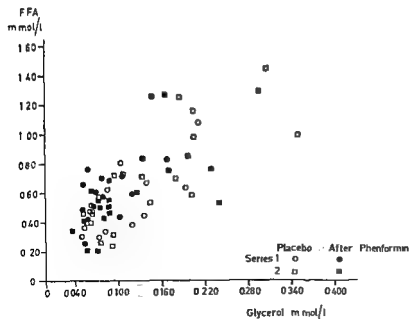


Fig 3 The correlation between the plasma concentrations of free fatty acids and glycerol in both series

documented case in Fig 1 the conclusion seems warranted that with the given dose phenformin has a weight reducing effect in cases of moderate obesity

Roginsky and Barnett (12) observed a weight loss, in patients on phenformin of the same order of magnitude as that obtained in the present study although their results were only significant for patients with a family history of diabetes

The reducing effect observed also agrees with the preliminary reports of Burstein et al (2) and with those of Liebermeister et al (10) who found a mean weight loss of 1.4 kg in 35 obese patients after six weeks on a dosage of 200 mg of butyl biguanide. Moreover this effect is in keeping with Pedersen's (11) finding that weight loss increased when patients on 1250 calories were given 3-400 mg of dimethyl biguanide hydrochloride (metformin). Pedersen emphasized the need of a high dosage and it is possible that the patients in the second series who were stouter should have received more phenformin for a comparable dosage. When a weight reducing effect is observed in obese subjects given phenformin this seems to be of about the same order of magnitude as that in obese diabetics (5).

On caloric restriction leading to weight loss the acetone concentration in the alveolar air increases (13). The absence of changes in the acetone concentration in series II may be accounted for by the absence of weight changes. In series I the weight changes after placebo were also small whereas a certain increase in the acetone concentration would have been expected on phenformin judging by the weight loss. This lack of increase in the acetone concentration may be explained either by a reduced tendency to ketosis in obese subjects on caloric restrictions as described by Kekwick et al (6) or by an antiketogenic effect due to phenformin itself.

No changes in the concentration of FFA were observed after phenformin which agrees with the findings of Burstein et al (2) and of Liebermeister et al (10). The latter found no changes in the concentration of plasma glycerol whereas in the present study a statistically significant ($p < 0.01$, $n = 23$) decrease was observed during phenformin treatment. A more detailed comparison with the results of Liebermeister et al is not possible owing to the brevity of their report.

For reasons discussed by Havel (4) and Stein-

berg (15) the rate of lipid mobilization in adipose tissue is more adequately illustrated by the plasma concentration of glycerol than by FFA. The present results may therefore be explained by a reduction in the rate of lipid mobilization in the morning due to phenformin on the assumption that phenformin does not increase the glycerol elimination rate.

ACKNOWLEDGEMENT

The study was supported by Pharmacia AB, Uppsala, Sweden.

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PENTAERYTHRITOLTETRANICOTINATE (PERYCIT®) IN THE TREATMENT OF HYPERCHOLESTEROLAEMIA

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Abstract For more than ten years we have tried to attack the problem of hypercholesterolaemia and hyperlipaemia using dietary treatment as well as various drugs.

Nicotinic acid given per os has been shown to lower serum cholesterol. A rather high frequency of side effects has motivated trials of derivatives with less marked side effects.

Pentaerythritoltetramicotinate is equal in effect to nicotinic acid but produces fewer side effects. During treatment periods of up to six years no harmful influence was seen.

A comparison of the three substances clofibrate, triiodothyropropionic acid and pentaerythritoltetramicotinate indicates that clofibrate is effective in lowering cholesterol in most cases. No effect on peripheral circulation has been observed and at least during short periods it does not combat angina pectoris.

Triiodothyropropionic acid is particularly effective in cases with a tendency to hypothyroidism. Its availability is reduced by a tendency to produce or increase angina pectoris. Pentaerythritoltetramicotinate Perycit® is equal in effect and better tolerated than nicotinic acid but some patients may not tolerate it either. Perycit® is always effective in lowering cholesterol, it increases the peripheral circulation and has a favourable effect on angina pectoris.

The purpose of this paper is to report on the experiences gained from clinical trials with pentaerythritoltetranicotinate (Perycit®) in tablet form.

Chemistry

Pentaerythritoltetranicotinate (Perycit®) (trial name 8 AL AB Bofors Nobel Pharma Sweden) is an ester of nicotinic acid. The drug is sparingly soluble in water (0.034 mg/ml (37°C)) but more easily soluble in gastric juice (16.5 mg/ml (37°C)) (7).

Pharmacology

LD₅₀ orally in mice is >10 000 mg/kg. The drug has passed toxicity tests performed on rats and dogs. Tests for possible teratogenic effects in rats and rabbits proved negative for therapeutic doses.

Compared with the same dosage of nicotinic acid 8 AL produces less flushing in the guinea pig (neck and ear)

In rabbits 8 AL has been shown to have a better protective effect, than nicotinic acid against arteriosclerosis induced by diet rich in fat and cholesterol (9).

First Trial (Long Term)

MATERIAL AND METHODS

Nicotinic acid was first tried on 15 of the patients. Two of them could tolerate 1 g three times daily but 13 immediately declared that the drug produced such an intense flush often accompanied by dizziness that it had to be withdrawn. When given 8 AL 1 g × 3 three of these 13 patients stated that they could not tolerate this preparation either. In the remaining ten patients similar but milder reactions occurred as with nicotinic acid and they could red 8 AL tolerable.

Seventeen patients including the ten just mentioned received treatment with 8 AL for not less than two months. Treatment was temporarily stopped for one to two months. Today six of the patients have been on 8 AL for five to six years. In addition to hypercholesterolaemia three of the patients had hyperlipaemia, six had earlier cardiac infarctions and 11 suffered from angina pectoris without electrocardiographic confirmation of coronary insufficiency. All the patients were prescribed a low fat diet with a high ratio of polyunsaturated fatty acids. The patients followed the instructions but admitted to some transgressions especially at Christmas.

During the whole course of treatment all the patients were regularly examined at first once a week later monthly to quarterly with blood tests including white cell differential and thrombocyte counts, serum tests for liver function on cholesterol and protein, ECG etc.

RESULTS

Less than 3 g daily of 8 AL did not lower the serum cholesterol level permanently. With a dosage of 3-6 g daily a permanent fall was obtained. The decrease occurred after one to two weeks of therapy. If 8 AL was withdrawn the cholesterol returned to its original level in two weeks. This occurred even after several years of treatment.

Table II Results of bromsulphalein retention tests and liver biopsies

Pat no	Sex	Age	Bromsulphalein retention (%)	8 AL dose (g)	Period of treatment	Liver biopsy
3	♂	70	9.4	4-5	6 mo	No signs of marked liver damage
3	♂	70	6.0	4-5	7 mo	
3	♂	70	22.8	0	2 d	
7	♂	44	4.3	3	8 mo	
12	♀	41	16	6	4 mo	
12	♀	41	4.1	6	4½ mo	No changes of the kind observed following damaging medication
12	♀	41	1	11	3 w	
12	♀	41	2	10	2 mo	
13	♀	50	1	4	5 mo	
14	♀	49	10.8	3	4½ mo	
14	♀	49	5.6	3	6 mo	No pathological signs
15	♂	58	8.8	4	2 w	
16	♀	57	6.3	3	18 mo	
17	♂	62	1.1	3	13 mo	
18	♂	59	7.6	4	2 mo	
19	♀	60				Reaction (cf text)

ing could be accompanied by dizziness. Some patients remarked that if they ate something acid, usually oranges between meals, this could promptly cause flushing, even some hours after taking the tablets.

Gastrointestinal complications

Usually slight nausea and occasionally slight anorexia occurred periodically when large doses were administered for a long time. These complications were comparatively easy to treat with antacids, sometimes in combination with anticholinergics. Gastric symptoms were most marked in patients with an earlier tendency to gastritis.

Two patients had a myocardial infarction during treatment. After recovery treatment with 8 AL continued for more than two years. In neither case was the 8 AL therapy considered to have caused the infarction.

Second Trial

MATERIAL AND METHODS

The second group studied consisted of 12 outpatients with hypercholesterolaemia and angina pectoris (Table III). Two patients (1 and 3) from the long-term trial were temporarily included.

The trial was divided into four periods:

- 1 A control period of four weeks with the same dietary instructions as in the long-term trial and no further therapy.
- 2 A treatment period of six weeks during which 8 AL was added.
- 3 A second control period of six weeks without this drug.
- 4 A further treatment period of six weeks.

Table III Distribution of patients

Pat no	Sex	Age	Weight (kg)	Chol (mg)	Diagnosis other than hypercholesterolaemia and angina pectoris
1	♀	59	79	352	
2	♀	77	72	333	Benign essential hypertension
3	♂	70	64	390	Myxoedema
4	♀	60	67	339	Spondylos deformans, hypertension, cystitis
5	♂	61	83	347	Benign essential hypertension, Diverticulitis coli
6	♀	55	87	383	Benign essential hypertension, Myxoedema
7	♂	44	58	388	Hypertension, Myocardial infarction
8	♀	53	48	431	Hypertension, Acute myelitis
9	♀	36	60	425	Hypertension
10	♂	58	77	355	Hypertension, xanthoma (olecranon region)
11	♀	65	4	349	Hyperlipaemia, Raynaud's phenomenon
12	♀	41	66	369	Hyperlipaemia, Coronary insufficiency, Intermittent claudication

Mean values from control period 1

Table IV Mean values of cholesterol in mg

Pat no	Control period 1	Treatment period 1	Control period 2	Treatment period 2
1	352	243	311	260
2	333	298	319	281
3	390	350	433	304
4	339	310	361	321
5	347	316	359	302
6	383	417	455	337
7	388	229	315	264
8	431	294	329	251
9	45	311	415	349
10	355	216	331	—
11	394	265	—	—
12	369	360	—	—
Mean ^b ± s.e.	376 ± 12	308 ± 18	366 ± 18	294 ± 11

t test on individual differences^bControl period 1 v treatment period 1 $p < 0.05$ Control period 2 v treatment period 2 $p < 0.001$ Control period 1 v control period 2 $n.s.$ Treatment period 1 v treatment period 2 $n.s.$ ^a Dosage only 1.5 g daily^b Patients no. 1-9

In some instances the second control period was extended without affecting the trial. The dosage of 8 AL was 3 g daily.

Serum cholesterol determinations (6-11) were made every second week, thus providing three values per period. Liver function was followed by serum bilirubin levels, alkaline phosphatases, thymol turbidity and SGOT. Leucocyte and platelet counts as well as prothrombin estimations were made.

RESULTS

The effect of 8 AL on the serum cholesterol is shown in Table IV and Fig. 2. With adequate dosage a statistically significant lowering of the serum cholesterol level was obtained. Seven patients were not on diet when the trial started. Before the first control period they had a mean cholesterol value of 420 ± 22 mg%, which decreased to 378 ± 16 mg% during this period.

All patients reported that their angina pectoris was both less severe and less frequent and five of them considered that the sensation of heaviness in their legs was milder when taking 8 AL.

Studies have shown that nicotinic acid is more rapidly eliminated from the body than 8 AL (14). The advantage of slow elimination is that a lower dosage ought to produce an adequate lowering of the cholesterol level. This may be illustrated by

patient 12 who prior to the present investigation was given in a trial nicotinic acid in increasing doses up to 60 g daily whereby her cholesterol level fell from 370 mg/100 ml to 291 mg/100 ml. When a steady cholesterol level had been established a change to an equal dose of 8 AL resulted in a further fall to 212 mg/100 ml. A parallel lowering of her serum lipids was obtained. These results were reproduced with a somewhat lower dose. During this trial the patient complained of marked gastric symptoms when on nicotinic acid which were alleviated only to a minor degree by antacids. When she received 8 AL her symptoms rapidly subsided. While on 8 AL therapy she also reported improved walking capacity.

Laboratory investigations

Laboratory results are shown in Table V. Patient 1 showed a rise in serum alkaline phosphatases. These were rapidly normalized after the end of the trial and were probably elevated owing to gallbladder disease. Patient 12 showed already before the trial a positive thymol turbidity reaction of 0.14 which remained unchanged. Triglycerides were also determined but not regularly; they followed the variations in serum cholesterol.

Side effects

Side effects were of the same kind and frequency as in the long term trial. Thus flushing was recorded in all the patients except no. 12. In one case flush and in two hyperacidity caused therapy to be discontinued.

DISCUSSION

The effect of nicotinic acid in lowering serum lipids is amply documented (1, 2, 3, 9, 15, 16, 21). Three g or more daily of 8 AL also produce a reduction of the same magnitude in the serum

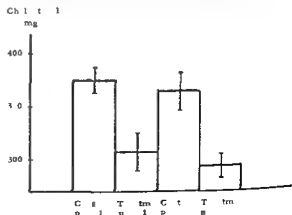


Fig. 2 Cholesterol values (mean ± s.e.) for patients no. 1-9.

Table V Values of different clinical and laboratory tests during the investigation

Pat #	Blood pressure		BMR		SGOT		Icterus index		Thymol turbidity		Alkaline phosphatase		Leuco- cytes 10 ³		Platelets 10		Pro- throm- bin	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
1	155/90			±0	26	26	1 4	1 1	0.03	0.05	7 4	12 7	2 9	4 3	266	196	96	
	160/110	170/110			20	20	1 5	1 6	0.04	0.06	4 0	4 0	4 4	4 3	288	234	111	77
3	160/80		-1		15	30	1 5	1 3	0.07	0.12	1 6	2 0	5 8	4 3	168	222	105	79
4	130/80		+2		17	18	1 3	1 4	0.03	0.02	10 0	8 7	6 8	10 5	350	290	98	91
5	150/85	140/90	-4		28	17	1 5	1 5	0.03	0.04	7 9	5 8	7 7	5 4	350	346	104	110
6	170/100		+6		19	14	1 6	1 3	0.01	0.02	9 9	9 9	4 4	4 5	266	236	116	87
7	145/100		-1			22		1 8	0.06	0.05	5 8	6 2	4 4	3 8		208		
8		170/110	+12		12	17	1 5	1 8	0.05	0.06	5 2	7 5	3 9	3 6	170	224		
9	140/80	145/90	-2		26	34	1 5	1 7	0.07	0.09	8 6	8 3	4 4	4 2	244	172		
10	135/80		-14				1 6	1 3	0.04	0.03	4 5	7 9	5 6					
11	150/80		-4		12	19	1 5		0.01	0.02	3 6		5 3					
12	140/85		+8		14	16	1 6	1 3	0.14	0.16	4 1	4 8			264			

C = values towards the end of a control period
T = values towards the end of a treatment period

cholesterol. As with nicotinic acid this decrease is reversible when therapy is withdrawn.

Nicotinic acid in the requisite doses for an effective lowering of serum cholesterol i.e. 3 g a day or more (16) causes such pronounced side effects at least in the earlier stages of treatment that the patients often refuse to continue the therapy (5, 13, 17, 19). The side effects can be reduced by taking the drug after meals and by administering successively increasing doses. According to the patients the side effects of 8 AL were considerably milder and more tolerable.

Flush is the most troublesome of the side effects. Generally it subsides after a short period of treatment but some patients insist that it is impossible to raise the dose beyond a certain limit—commonly 0.5 g each time. It is possible to achieve higher dosage by dividing the dose into several small doses. Stomach complaints such as generally occur in connection with gastritis can be coped with by administration of antacids or/and anticholinergic drugs.

Modifications of nicotinic acid with a prolonged effect may be associated with liver damage (10, 20). Regular controls of blood and liver function (bilirubin, thymol turbidity, alkaline phosphatases and SGOT) have not shown any serious side effect even in patients who have taken 8 AL 3 g a day for 5–6 years.

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THE EFFECT OF PROPRANOLOL ON ECG IN ANGINA PECTORIS AND ORTHOSTATIC TACHYCARDIA¹

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Abstract The effect of intravenous infusion of 5 mg propranolol on the electrocardiogram at rest during and after work on a bicycle ergometer was studied in 26 patients with either typical or questionable coronary heart disease or with orthostatic tachycardia. In the last group electrocardiograms were also registered in the upright position. In the patients with orthostatism propranolol elicited a normalization of the S-T and T changes in standing position and during work. Propranolol had no demonstrable effect on the electrocardiographic picture during work in patients with typical coronary disease though it did elicit a significant resolution of the depressed S-T and T segments three minutes after work. This observation may be connected with the possibility that the cardiac work and hence the degree of ischemia was less in the work test after propranolol compared with that before. In the patients with questionable coronary disease propranolol elicited a reduction of the electrocardiographic changes both during and after work. Such an effect would be in line with the presence of an increased activity in the cardiac sympathetic nerves and possibly also with a moderate degree of coronary sclerosis.

Attempts to differentiate between a vegetative and an organic etiology of electrocardiographic changes using sympatholytic agents were made at an early stage (2, 17, 21). Besides blocking alpha receptors, however, the substances available at the time also stimulated adrenergic receptors making it difficult to assess the results. Interest in this type of differential diagnostic study has revived with the advent of substances chiefly propranolol that specifically block beta adrenergic receptors and thus the adrenergic functions of the heart. Thus it has been shown that S-T and T changes connected with orthostatic reactions can be suppressed by treatment with propranolol (7, 13, 18). This observation was held to confirm the previous assertion that electrocardiographic changes of this type indicate an elevated sympathetic tone in the heart.

Propranolol has also been used to differentiate electrocardiographic changes caused by functional or organic cardiac disorder observed after effort. It has thus been found that in patients with coronary disease propranolol or its predecessor pronethalol does not affect S-T and T changes that arise during work (7, 22) or after work (23). Furberg (7) reported however that the electrocardiographic changes became normalized more rapidly after the end of work when patients with coronary disease were given propranolol. He interpreted this to mean that the shape of the S-T segment after the end of work is influenced not only by ischemic changes but also by changes in the activity of cardiac sympathetic nerves.

The present investigation was an attempt to evaluate the use of propranolol for the assessment of S-T changes on exercise electrocardiograms. The investigations concerned the effect of the substance upon electrocardiographic changes during and after work in patients with clinically established or suspected coronary insufficiency as well as in individuals with orthostatic reactions.

METHODS

Electrocardiography

Electrocardiograms were recorded at rest in both the supine and the standing position using unipolar and bipolar extremity leads as well as the following chest leads CR, CR₁, CR₂, and CR. Only the chest leads were used during work; the indifferent electrode being shifted from the right arm to the forehead (CH). This change does not usually affect the pattern of the electrocardiogram (11).

The electrocardiograms were coded according to Black

A report on an introductory study was presented at the Swedish Medical Convention (Mediska Riksstämman) in Stockholm 1964.

Table 1 Heart rate (means \pm SE) at rest during and after exercise performed before and after intravenous infusion of 5 mg propranolol

The data given during work (before and after propranolol) were obtained at identical times during the same work load. The heart rates just before the end of work were not always obtained at identical work loads in each patient before and after propranolol (see Procedure).

Patients	Rest		Work (200–300 kpm/min)		Work (400–600 kpm/min)		Just before the end of work		3 min after work	
	Before	After	Before	After	Before	After	Before	After	Before	After
With typical coronary heart disease ($n=12$)	72.4	63.5	100.2	88.3	127.8	109.5	133.8	118.2	81.2	73.8
	± 2.7	± 2.1	± 2.7	± 2.5	± 3.0	± 2.7	± 4.8	± 3.5	± 2.8	± 2.2
With questionable coronary heart disease ($n=6$)	76.5	66.0	112.7	95.5	147.0	119.8	148.5	131.4	85.9	76.1
	± 5.9	± 3.5	± 6.6	± 8.1	± 16.6	± 13.7	± 11.4	± 13.1	± 6.0	± 5.1
With orthostatic tachycardia ($n=8$)	75.0	65.6	114.8	91.8	140.7	112.1	153.6	134.0	92.5	78.1
	± 2.3	± 2.1	± 5.3	± 3.3	± 6.1	± 3.3	± 9.2	± 7.3	± 6.6	± 4.6

burn III a1 (3) as modified by Astrand (1) and with two further modifications whereby IV 7 stands for isoelectric or elevated S-T with normal configuration and an additional category V 4 is added under V. The code employed was as follows:

- IV 1 S-T depression 1 mm or more and S-T segment horizontal or downward sloping
- IV 2 S-T depression 0.5–0.9 mm and S-T segment horizontal or downward sloping
- IV 3 No S-T depression as much as 0.5 mm but S-T segment sloping down to 0.5 mm or more below PR baseline
- IV 4 No S-T depression as much as 0.5 mm S-T segment horizontal or downward sloping but not reaching 0.5 mm below PR baseline
- IV 5 S-T depression 1 mm or more with normal configuration of S-T segment
- IV 6 S-T depression 0.5–0.9 mm with normal configuration of S-T segment T wave stems (when R amplitude = 5 mm or more in aVL and QRS mainly upright in aVF)
- IV 7 Isoelectric or elevated S-T with normal configuration
- V 1 T amplitude = minus 5 mm or more (I II CR–CR₂)
- V 2 T amplitude = minus 1 to 5 mm (I II CR₂–CR₃)
- V 3 T wave flat or slightly diphasic negative phase less than 1 mm (I II CR–CR₂)
- V 4 T amplitude positive 2 mm or more (I II CR₂–)

PATIENTS

Twenty-six patients participated in the investigation. All showed ST-T depressions on electrocardiographic recordings during exercise. They were divided into three groups.

Group 1 (patients with typical coronary heart disease). Twelve patients (10 men and 2 women) had symptoms of effort angina with pain located retrosternally or to the left side of the chest radiating to the left arm, and with relief of pain at rest and after treatment with nitroglycerin.

Group 2 (patients with questionable coronary heart

disease). Six patients (3 men and 3 women) experienced no pain on exercise. However their electrocardiographic changes during exercise were similar to those in group 1. The possibility that these patients had a coronary disease could therefore not be ruled out.

Four of them (2 men 54 and 57 years and two women 32 and 47 years) had pain located to the left side of the chest (in two of them radiating to the left arm) which could appear at any time with no relation to exercise. In two of them coronary angiography showed no abnormality except a suspected narrowing of a vessel in one of them. Furthermore this patient had almost consistently atrial fibrillation. The remaining two patients had no history of pains. They included one man 51 years with arteriosclerotic changes of the aorta as shown at angiography and one woman 58 years with diabetes mellitus.

Group 3 (patients with orthostatic tachycardia). The remaining eight patients (5 men and 3 women) had no angina pectoris. However they developed extensive tachycardia and electrocardiographic ST-T depression during 8 min standing.

The ages (means \pm s.e.) of the patients were 51 ± 3 in group 1, 50 ± 4.0 in group 2 and 35 ± 3.6 years in group 3.

PROCEDURE

The work test was performed on an electrically braked bicycle ergometer (9). The load was increased successively the initial level being 200 kpm/min for women and 300 (in a few cases 200) kpm/min for men.

Two identical studies were performed within a week on each individual. The studies were made on different days in the case of eighteen patients; in the other eight they were performed on the same day with at least two hours rest between the exercise tests (two of these nine patients belonged to group 1, two to group 2 and four to group 3). On each occasion electrocardiograms were recorded at rest in the supine position and also in the case of patients with orthostatism after 8 min standing. The work test was then performed in the sitting position electrocardiograms being registered after 2, 4, 5 and 6 min work at each load as well as just before the end of work.

The patient was then placed in the supine position and a further registration was made 3 min later. On the second occasion 5 mg propranolol (Inderal) was infused intravenously during 5 min into the supine patient (Inderal from Dr A. Wagner AB, Scania AB, Göteborg, Sweden.) Further electrocardiograms were then registered 5 min after the end of the infusion followed by the orthostatic test and the exercise test with electrocardiographic recordings as on the first occasion. The load was increased every sixth minute by 200 and 300 kpm/min in women and men respectively. The work test was always continued until the patient experienced angina pectoris or fatigue. This meant that the total amount of work performed was not always the same before and after propranolol. The second work test was completed about half an hour after the infusion of propranolol.

RESULTS

Heart Rate

As will be seen from Table I, propranolol elicited a fall in heart rate at rest in all groups as well as a reduced rise in heart rate during work. The heart rate measured at the end of work was also lower after propranolol even though the total amount of work performed was somewhat larger. Furthermore, the heart rate 3 min after the end of work was lower after propranolol. The patients with orthostatic tachycardia displayed during standing a lower increase in heart rate after propranolol than before, the means \pm SE being 102.3 ± 5.1 before and 78.9 ± 3.4 after propranolol ($p < 0.01$).

Electrocardiograms (Fig. 1)

Group 1 (patients with typical coronary heart disease)

Before propranolol the S-T and T segments at rest showed only moderate deviations from the normal picture in all cases and the appearance of the S-T segment was never classified below IV 4. Propranolol reduced these changes in a few cases but the difference was not significant for the group as a whole.

During work all patients displayed a marked depression of the S-T before propranolol in many cases combined with a flattened or diphasic T. The electrocardiographic picture was not influenced by propranolol either when the comparison was made at the same work load or at the same heart rate.

After the end of work the S-T changes recorded were significantly smaller after propranolol than before. The T changes were also reduced to some extent by propranolol.

Group 2 (patients with questionable coronary heart disease)

All the electrocardiograms registered at rest in this group displayed only very slight or no deviations from the normal picture.

During work there was moderate to marked depression of the S-T segments while the T wave remained practically within normal limits. The infusion of propranolol was followed by less marked S-T changes though the difference was not significant.

After work the S-T depression before propranolol was of approximately the same magnitude as during work. Propranolol resulted in an almost complete normalization (Fig. 1).

Group 3 (patients with orthostatic tachycardia)

All the electrocardiograms in this group displayed normal or almost normal S-T and T segments at rest.

In the upright position all cases displayed depression of the S-T segment and a flattened or diphasic T (Fig. 2). A normalization was registered throughout after propranolol; the difference in the electrocardiographic reaction before and after the infusion being significant.

During work the electrocardiographic picture was always essentially similar to that recorded in the standing posture with a rapid normalization immediately after the end of work. Propranolol normalized the electrocardiographic reaction during work.

Physical Work Capacity

In the group of patients with typical coronary heart disease the mean maximum load tolerated by the men was 600 kpm/min, the duration of work at this load being on an average 3 min before and 5 min after propranolol. For the women the average work capacity was 400 kpm/min for 1 min before and for 3 min after propranolol. These differences are not significant. In the group of patients with questionable coronary heart disease the corresponding data for the men were 600 kpm/min for 5 min before and for 6 min after propranolol and for the women 400 kpm/min for 6 min before and 600 kpm/min for 1 min after propranolol. These differences are not significant either. For the individuals with orthostatic tachycardia the average maximal load tolerated before propranolol was 1200 kpm/min for 1 min.

S-T CLASSIFICATION

T CLASSIFICATION

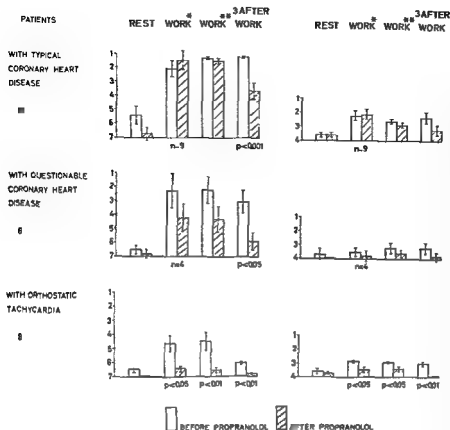


Fig 1 S-T and T classification of electrocardiograms at rest during work and 3 min after work in patients with 1 typical coronary heart disease 2 questionable coronary heart disease and 3 orthostatic tachycardia before and after intravenous infusion of 5 mg propranolol (means \pm s.e.) S-T classification 7=normal configuration T classification 4=normal configuration (see Methods)

* Comparison between the work load before propranolol that gave the same heart rate as the highest load after propranolol

** Comparison between the same work load (highest comparable load) before and after propranolol

for the men and 400 kpm/min for 6 min for the women. After propranolol the corresponding \dot{V}_{O_2} was 1200 kpm/min for 2 min and 600 kpm/min for 2 min. Neither difference is significant.

S-T CLASSIFICATION

T CLASSIFICATION

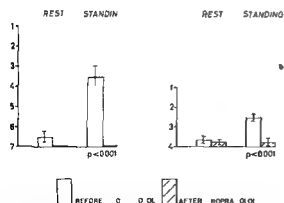


Fig 2 S-T and T classification of electrocardiograms at rest and standing in patients with orthostatic tachycardia before and after intravenous infusion of 5 mg propranolol (means \pm s.e.) S-T classification 7=normal configuration T classification 4=normal configuration (see Methods)

Subjective discomfort during work

In group 1 the physical working capacity was limited in all cases by unquestionable complaints of angina pectoris. None of these patients reported any essential relief from the administration of propranolol. One patient who had emphysema in addition to coronary insufficiency reported much more marked dyspnea during the work test after propranolol. In group 2 the working capacity was limited by feelings of general fatigue or by uncharacteristic complaints from the chest. Propranolol did not significantly affect the complaints in this group either. Finally in the orthostatic group the limiting factor was fatigue chiefly in the leg muscles. Three of these individuals reported a less pronounced sensation of fatigue at the same load after propranolol.

DISCUSSION

In routine clinical work one is often faced with the problem of whether in the individual case electrocardiographic changes in the ST-T segment

at rest or in connection with physical work reflect ischemic cardiac disease or whether they are of a more functional nature. It seems difficult for theoretical reasons to determine the underlying cause of an ST-T change solely from the appearance of the electrocardiogram. The disturbance in the electrolyte balance at the cellular level which is probably the ultimate cause of the repolarization disturbance can thus be elicited by such widely different factors as ischemia, drugs and hypokalemia. Lepeschkin, however, has reported a flattened and depressed ST segment caused by an injury current of the subendocardial muscle fibres as a result of true coronary insufficiency (14). It has therefore been considered that such an ST change, often combined with a diphasic T after work, constitutes a criterion of latent myocardial ischemia (24). Reference has thus been made in this context to the similarity between this change and that which may appear at rest in connection with an attack of angina pectoris. A good correlation has also been demonstrated between this type of electrocardiographic change and the mortality in coronary disease (16, 20). On the other hand, individuals with pronounced autonomic disturbances but not demonstrable cardiac disorder (referred to as vasoregulatory asthenia) may sometimes display a similar electrocardiographic picture during work with normalization immediately after the end of work (10). Also in coronary heart disease the ST-T changes are most pronounced during work (4). From this it follows that one cannot always differentiate between organic and functional cardiac disorders simply on the basis of the type of electrocardiographic changes or the conditions under which these occur.

It would therefore be valuable to have a substance that blocks the influence of the central nervous system upon the function of the cardiac muscle but which does not affect ST and T changes of organic origin. The present results support the observation by Furberg (7) that drugs which elicit adrenergic beta-receptor blockade may be of value for this purpose. A clear electrocardiographic difference was thus demonstrated between patients with pronounced orthostatism and patients with coronary heart disease. In the former group propranolol elicited a normalization of the ST and T changes in the standing position as well as during work, whereas no electrocardiographic effect could be demon-

strated during work in the group with coronary disease.

It is particularly worth noting that propranolol significantly reduced the ST and T changes after the end of work in patients with typical clinical symptoms of angina pectoris. This finding, which agrees with Furberg's observation (7), indicates that in patients on acute or chronic beta-adrenergic blockade the electrocardiographic analysis must refer to conditions *during* work when evaluating the ST and T regions in cases with suspected ischemic heart disease. An analysis confined to electrocardiographic changes *after* work may thus give a false negative picture in such cases.

It is not entirely clear why propranolol affects the electrocardiographic picture after the end of work but not during work in these cases. According to Furberg (7) this indicates that after the end of work functional ST and T changes may be added to those of organic origin. In the majority of individuals with coronary disease the ST and T changes during work and in the first few minutes afterwards are probably conditioned by the ischemic process. This is supported by the observation of Kaiser (12) that there is a good correlation between the intensity of work and the degree of ST and T changes during as well as after work in patients with coronary insufficiency. In the present study the ST changes at the end of work and three minutes after work were identical in all patients with clinically established coronary insufficiency. This tends to refute the presence of an additional component (i.e. a varying degree of autonomic tonus) behind the ST-T changes after work.

It is more likely that the reduction elicited by propranolol in the ST and T changes after the end of work was due to the ventricular load and hence to the degree of ischemia being less pronounced in the work test after propranolol. Thus in studies of how beta-adrenergic receptor blocking agents influence the central circulatory adaptation during work it has been shown that cardiac output, left ventricular work and ventricular ejection rate during work are all significantly lower after the administration of propranolol than before (5, 6, 8, 15, 19). In view of this the present patients were allowed—as far as their subjective experience of the work permitted—to perform a somewhat larger amount of work after the propranolol blockade than before. Even so there was no case in

which the heart rate after propranolol rose as high as it had during the first work test. It is thus conceivable that in spite of the greater amount of work performed the strain on the myocardium and hence the degree of ischemia were not as pronounced during the second work test as during the first. This is also suggested by the observation that the recovery from work as judged from the heart rate was somewhat more rapid after propranolol which has also been reported by Cumming and Carr (5).

In the group of patients with questionable coronary heart disease the electrocardiographic changes were reduced after propranolol not only after the end of work but also during work. It is possible that in cases with only a moderate deterioration in the coronary circulation the reduction of left ventricular work and hence the oxygen requirement of the myocardium induced by propranolol results in an improved electrocardiographic picture even during exercise whereas in more advanced cases this effect does not materialize until after the end of work. However the group of patients with questionable coronary heart disease included those with relatively unspecific symptoms and consequently the electrocardiographic changes may have been partly of a functional nature. The etiological background to these observations can only be elucidated by further

ACKNOWLEDGEMENTS

Grants for this study have been made available by the Swedish Medical Research Council, the Association of the Swedish Pharmaceutical Industry and the Swedish Association against Heart and Lung Diseases.

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CAPILLARY PERMEABILITY SURFACE AREA PRODUCT (PS) OF RENKIN
IN HUMAN SKELETAL MUSCLE*Effect of Locally Applied Norepinephrine*

PRELIMINARY REPORT

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The transcapillary exchange of water soluble substances is dependent upon the permeability of the capillary wall to the substance in question (P) and the surface area of the capillary bed open to flow (S) (4). Transcapillary exchange expressed as the clearance of a substance (C_i in ml whole blood/min/100 g tissue) from blood to tissue or vice versa is a function of blood flow (Q in ml whole blood/min/100 g) and PS (ml/min/100 g) the permeability surface area product or capillary diffusion capacity introduced by Renkin (7) which is the flux across the capillary surface per 100 g tissue at unit concentration difference (with concentrations referred to whole blood). Thus

$$C_i/Q = 1 - \exp(-PS/Q) \quad (1)$$

In contrast to water soluble substances the transcapillary exchange of small lipid soluble substances (e.g. xenon) is limited only by blood flow. This clearance thus measures capillary blood flow in all flow rates (5). Clearance is also the product of the clearance constant k_i and the partition coefficient between blood and tissue λ . Thus

$$C_i = k_i \lambda_i \quad (2)$$

where C_i is expressed in ml/min/100 g. Combining equations 1 and 2 and assuming that $Q = C_x$ (clearance of xenon) yields

$$\frac{k_i}{\lambda_x} = \frac{\lambda_x}{\lambda_i} \left[1 - \exp\left(-\frac{PS}{k_x \lambda_x \cdot 100}\right) \right] \quad (3)$$

A detailed derivation of formula 3 with special reference to transport from tissue to blood is given elsewhere (3). We have applied this formula to the calculation of PS in human skeletal muscle

at rest by measuring the simultaneous tissue clearance of ^{24}Na and ^{133}Xe (5). λ_x is 0.7 for skeletal muscle at normal hematocrit (2). The sodium space of skeletal muscle is 13% (1). Assuming that Na is extracellular and has approximately equal concentrations in interstitial space and plasma λ_x at normal hematocrit is about 0.24. With these data it is then possible to calculate PS . k_x/λ_{x0} approaches λ_x/λ_i as λ_i (or k_x) approaches zero. Error in the assumed value of λ_x/λ_{x0} changes the y intercept of the PS curves (see Fig. 1) and the absolute values of PS but changes neither their general form nor the physiological conclusions.

Lassen (6) has recently applied an approximate formula to the calculation of maximal diffusion capacity in exercising muscle which differs from PS by (1 Hematocrit) and by the approximation of a fairly low extraction from tissue to blood at high flows.

PS in resting muscle of healthy adult volunteers was 1.52 ± 0.46 ml/min/100 g (35 injections in 13 subjects) (Fig. 1). With 0.04 mg/ml norepinephrine locally (42 injections in 13 subjects) PS was 0.46 ± 0.20 ($P < 0.001$). Note the change in the shape of the PS curve illustrating that equal capillary blood flow does not necessarily mean equal transcapillary exchange.

The analytical procedure described here affords an opportunity for relatively easily obtaining data of fundamental physiological importance concerning the capillary bed in human skeletal muscle. Characterization of the transport function of the capillary bed in clinical situations not only at maximum transport rates but also with a capillary bed of normal or less than normal

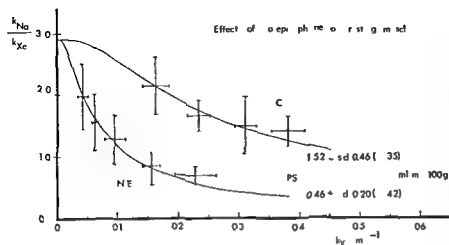


Fig. 1 k_{Na}/k_{Xe} vs k_{Xe} as classed mean values following simultaneous intramuscular injection of Na and ^{133}Xe in human tibialis anterior muscle. C—at rest, N.E.—at rest with addition of norepinephrine. The solid lines were calculated from formula 3 with the mean PS of the 2 groups.

capacity is stressed. We are primarily interested in changes of PS at subphysiological flows such as can be seen in shock, hemorrhage and trauma. Such studies are now in progress both in experimental shock models and in severely ill patients.

ACKNOWLEDGEMENTS

This work has been supported by grants from the Swedish Medical Research Council (Contract No. Y-447) and from the United States Army through its European Research Office (Contract No. DAJ437 67 C 0460).

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GLUCOSE TOLERANCE PLASMA LIPIDS AND SERUM INSULIN IN PATIENTS WITH ISCHAEMIC HEART DISEASES

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Abstract The glucose tolerance the insulin concentration in serum and concentrations of free fatty acids (FFA) triglyceride and cholesterol in plasma or serum were examined in 75 patients of normal weight and below the age of 67 years they were all suffering from ischaemic heart diseases (angina pectoris and/or earlier episodes of coronary occlusion). A clinical diagnosis of diabetes mellitus was not established in any of these cases the patients were not predisposed to diabetes and neither hypertension nor reduced renal function was observed in any of the patients. Patients who on an earlier occasion had experienced episodes of coronary occlusion were seen at least three years after such occurrence.

Among the 25 patients the glucose tolerance was reduced in 12 ($k < 110$) determinations being made by glucose elimination after intravenous application of glucose. In patients in whom the glucose tolerance was found to be reduced concentrations of insulin were reduced and FFA elevated suggesting latent diabetes mellitus. In patients in whom the glucose tolerance remained normal the insulin concentration in serum was found to be abnormally high.

Whether the demonstrated abnormal metabolism may be of significance for the pathogenesis of ischaemic cardiovascular lesions remains to be defined.

The increased incidence of ischaemic heart diseases in patients with diabetes mellitus has been ascertained beyond doubt on the basis of autopsy findings and clinical observations (9, 10, 11, 17, 18, 19, 20, 36, 41). Furthermore in recent investigations the glucose tolerance has been found to be reduced in up to 50 per cent of patients with ischaemic heart lesions in whom clinically recognizable symptoms of diabetes were absent (1, 4, 13, 15, 24, 26, 30, 31, 34, 35, 39, 40, 42). It is generally admitted however that glucose tolerance may be influenced by a number of factors. Consequently the present study was designed with a view to 1) defining whether a reduced glucose tolerance in patients with ischaemic heart lesions

might be demonstrable also in individuals in whom factors known to affect the glucose tolerance were absent such as hereditary predisposition old age (6, 23), obesity (43), hypertension (5, 25, 44), reduced renal function (37), medication by diuretics or steroids and recovery from earlier episodes of coronary occlusion (33, 34) and to 2) examining whether such decreased glucose tolerance coincides with changes in concentrations of insulin and lipids in serum analogously with changes seen in cases of frank diabetes mellitus.

MATERIAL

The material comprises 75 patients (2 women, 23 men) without clinically recognizable diabetes who at the time of examination were suffering from ischaemic heart lesions. In nine of the patients characteristic angina pectoris in relation to effort was manifest, coronary occlusion had occurred in the remaining 66 patients at least three years before the present examination. The diagnoses were in all cases verified by characteristic changes seen by electrocardiography and the elevated concentrations of enzymes in serum. At the time of examination the patients concerned were not hospitalized they were not even confined to bed. All patients received treatment by anti-coagulants but otherwise they had no medical or dietetic treatment. The following criteria were fulfilled in all cases: age below 65 years, weight within the range ± 10 per cent Danish average weight (Hafslund (Danish Life Insurance Company) statistics 1958), arterial blood pressure below 170/105 mm Hg, serum-creatinine concentration below 110 mg/100 ml, absence of congestive heart failure. There was no family history of diabetes in any of the cases.

METHODS

Intravenous glucose tolerance tests according to Lundbäck's method (1) and electrocardiography were carried out in all cases. The glucose concentration in capillary blood was determined according to Hagedorn's and Nor-

Table I *I.v. glucose tolerance test, fasting blood sugar, triglycerides and cholesterol in patients with ischaemic heart disease*

No	Age	Sex	Cardiac pain	Coron. occi.	Per cent of Danish mean body weight (Hafnia)	Δ value (Normal ≥ 1.10)	Fasting blood-sugar (mg/100 ml) (Normal = 80-120)	Triglycerides (mmol/l) (Normal = 0.46-1.98)	Cholesterol (mg/100 ml) (Normal = 170-240)
<i>Cases with reduced glucose tolerance</i>									
1	56	♂	+	0	99	0.41	127	1.69	275
2	52	♂	+	+	110	0.77	80	1.91	260
3	58	♂	+	0	106	0.83	114	0.48	239
4	61	♂	+	0	94	0.85	108	0.59	292
5	49	♂	+	+	87	0.87	116	1.33	197
6	48	♂	+	0	110	0.90	98	1.16	232
7	57	♂	+	+	101	0.91	104	0.42	301
8	60	♂	+	+	86	0.92	69	0.14	250
9	62	♂	+	0	104	0.97	106	1.50	285
10	41	♂	0	+	107	1.00	91	1.16	355
11	60	♀	+	0	89	1.03	97	0.97	320
12	58	♂	+	0	86	1.08	63	0.67	212
Mean	55				98.3	0.879	97.8	1.00 ^a	269.9

man Jensen's method (15) at intervals of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 min after intravenous application of 25 g of *D*-glucose. Concentrations of insulin in serum and of free fatty acids (FFA) in plasma were determined prior to application of glucose and subsequently at intervals of 10, 30, 60 and 120 min after the application. Concentrations of total cholesterol and triglyceride in serum were determined while the patients were in the fasting state.

The insulin concentration was determined immunologically in heparinized serum using the double antibody tech-

nique suggested by Hales & Randle (7) (¹²⁵I) labelled insulin from the Niels Steensen Hospital). FFA was determined according to Dole's method (8), the triglyceride concentration according to Laurell's method (16), the cholesterol concentration being determined according to Libermann-Burchardt's method (17). All analyses were carried out in duplicate. The analytical reproducibility given as the coefficient of variation (100 \times s/m) was as follows: glucose concentration 4.9%, insulin concentration 9.4%, FFA 5.0%, triglyceride concentration 3.0% and cholesterol concentration 3.6%.

Table II *I.v. glucose tolerance test, fasting blood sugar, triglycerides and cholesterol in patients with ischaemic heart disease*

No	Age	Sex	Cardiac pain	Coron. occi.	Per cent of Danish mean body weight (Hafnia)	Δ value (Normal ≥ 1.10)	Fasting blood-sugar (mg/100 ml) (Normal = 80-120)	Triglycerides (mmol/l) (Normal = 0.46-1.98)	Cholesterol (mg/100 ml) (Normal = 170-240)
<i>Cases with normal glucose tolerance</i>									
13	57	♂	-	0	94	1.11	98	1.69	239
14	61	♂	+	0	102	1.22	103	0.77	284
15	60	♂	0	+	94	1.27	87	0.84	287
16	53	♂	0	+	100	1.36	103	1.39	293
17	56	♂	+	+	108	1.44	60	2.23	221
18	60	♀	+	+	105	1.49	105	0.90	356
19	53	♂	+	+	93	1.51	75	1.09	4.1
20	40	♂	0	+	97	1.58	111	0.59	263
21	60	♂	0	+	105	1.59	87	1.61	377
22	43	♂	0	+	86	1.67	87	0.64	235
23	49	♂	0	+	86	1.87	89	1.44	346
24	60	♂	0	+	93	1.93	133	0.58	316
25	56	♂	+	+	94	2.01	86	2.96	374
Mean	54.6				96.7	1.542	94.3	1.288	309.8

The glucose tolerance was estimated on the basis of the slope (K) of the fall in the blood sugar concentration in a semi logarithmic system where $K = 100 \log 2/t_{1/2}$. The figure $t_{1/2}$ denotes the time in minutes until the glucose concentration is reduced by 50.

According to Wahlberg (47) normal values of glucose tolerance have been found to be $K \approx 1.10$. In our laboratory the following normal values were observed: fasting insulin concentration 15–9 $\mu\text{U/ml}$ (14), fasting FFA 770–934 mEq/l, fasting cholesterol concentration 170–40 mg/100 ml and fasting triglyceride concentration 0.46–1 mmol/l.

RESULTS

The findings appear from Tables I–III and from Figs 1–3. Among the 25 patients the glucose tolerance was found to be reduced in 12 ($K < 1.10$). An elevated fasting glucose concentration was observed in one of these patients and in one in whom the glucose tolerance remained normal.

Elevated fasting insulin concentrations in serum were seen in eight patients (Tables I and II) in six of the latter the glucose tolerance remained normal. Fasting insulin concentrations in serum were not found to be reduced in any of the patients.

Mean concentrations of insulin following injection of glucose were slightly higher in patients than in normal subjects (Fig 1). In patients with normal glucose tolerance the concentration of insulin was higher than concentrations observed in patients with reduced glucose tolerance (Fig 2 and Table III) and higher than those observed in normal subjects examined 10, 30 and 60 min after injection of glucose (Table III). In patients presenting reduced glucose tolerance the insulin concentration in serum was found to be significantly below normal level 10 min after injection of glucose and significantly higher 60 min after the injection (Fig 2 and Table III).

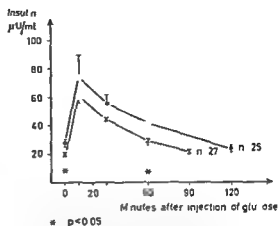


Fig 1 Insulin concentration (mean \pm S.E.) prior to and during intravenous glucose tolerance tests in young normal subjects (x—x) and in patients with ischaemic heart diseases (●—●).

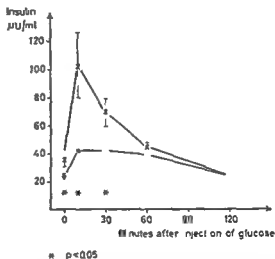


Fig 2 Insulin concentration (mean \pm S.E.) prior to and during glucose tolerance tests (GTT) in patients with ischaemic heart diseases: x—x patients with normal GTT ($n=13$); ●—● patients with reduced GTT ($n=17$).

Table III Insulin concentration (mean \pm standard deviation) before and during intravenous glucose tolerance test in young healthy people and in patients with ischaemic heart disease

	No	Fasting	10	30	60	90	120
A	7	19.6 \pm 5.1	60.7 \pm 22.0 ^a	44.0 \pm 12.0	9.2 \pm 9.9	21.6 \pm 5.7	
B	13	31.5 \pm 13.3 ^b	107.0 \pm 84.0 ^b	69.2 \pm 39.0 ^b	44.5 \pm 14.5 ^b		23.7 \pm 8.5
C	12	23.7 \pm 5.5 ^b	4.2 \pm 12.6 ^b	41.8 \pm 10.8 ^b	38.8 \pm 10.8 ^b		24.1 \pm 7.2

^a p for A versus C < 0.05

^b p for A versus B and B versus C < 0.05

A = normal healthy young people B = patients with normal glucose tolerance test C = patients with reduced glucose tolerance test

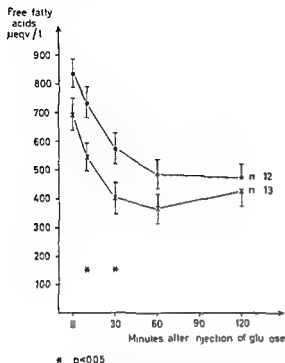


Fig 3 Free fatty acids (mean \pm SEM) prior to and during glucose tolerance tests (GTT) in patients with ischaemic heart diseases \times — \times patients with normal GTT ($n=13$) \bullet — \bullet patients with reduced GTT ($n=17$)

Elevated fasting FFA values were observed in five of the patients in one of these the glucose tolerance remained normal (Tables I and II). The fall in FFA values following glucose injection was less marked in patients with reduced glucose tolerance than in patients with normal tolerance the FFA values obtained 10 and 30 min after injection of glucose being significantly different (Fig 3).

In 20 of the patients the fasting cholesterol concentration was elevated (Tables I and II) among these the glucose tolerance remained normal in 11 cases. In two patients presenting normal glucose tolerance fasting triglyceride concentrations were elevated. Concentrations of cholesterol and of triglyceride were found to range at the same level in the two groups of patients with normal and reduced glucose tolerance respectively.

Only four out of the 25 patients presented normal concentrations of the three lipides examined the glucose tolerance was reduced in three of these patients.

A correlation between electrocardiographic changes, subjective symptoms and glucose tolerance was not demonstrable.

DISCUSSION

According to a report by Wahlberg (42) concerning a control material comprising patients without hereditary predisposition to diabetes and without hypertension obesity or ischaemic heart diseases the glucose tolerance was found to be reduced in 8% of all individuals in the age group 32 to 89 years.

As regards selection and composition the material here discussed conforms closely to the one reported by Wahlberg except that patients in the former group were suffering from ischaemic heart diseases. In the present material the incidence of reduced glucose tolerance was however six times as high as that observed by Wahlberg. Similar reduction of the glucose tolerance has been observed by several other authors in patients with ischaemic heart diseases (1, 4, 24, 26, 30, 31, 39, 42).

The reduced glucose tolerance can hardly be explained as a result of physical inactivity since all patients concerned had been up and about at the time of examination. It cannot be explained either as a result of the stress condition following coronary occlusion because the interval between the occurrence of the latter and the present examination was three years. Consequently the sign must presumably be co-existent with the ischaemic heart disease of the patients concerned.

In fact the patients seem to form two groups the glucose tolerance remaining normal in one group and being reduced in the other.

A characteristic of patients presenting reduced glucose tolerance was the less marked and late occurring rise in the insulin concentration in serum together with the relatively high FFA concentration following application of glucose. These features correspond closely to those seen in patients with maturity onset diabetes (22, 32).

The abnormally high insulin concentration in serum in patients with normal glucose tolerance whether in the fasting state or following glucose injections conforms with findings by Peters and Hales (28) and Nikkila et al (24) who studied patients with angina pectoris and patients with recent coronary infarction. According to these investigators and also to findings in the present material a normal glucose tolerance is maintainable exclusively if the organism manages to raise the insulin concentration in serum. Chlouverakis et al (3) observed that normal glucose tolerance

could not be maintained unless the insulin concentration in serum rose in parallel with increasing age. Whether the presence of insulin antagonists (38) or inhibition of the glucose transport owing to the vascular lesion is responsible remains to be defined.

The absence of a correlation between cholesterol or triglyceride concentrations in serum and glucose tolerance is in accordance with findings reported by Carlson and Wahlberg (2).

Thus the previously observed co-existence of ischaemic heart lesions and reduced glucose tolerance has been found in the present study to be valid also in cases of non hypertensive patients of normal weight in whom the renal function remains normal, patients who have never experienced episodes of heart failure and who do not receive diuretic drugs. In addition the reduced glucose tolerance was found to be accompanied by changes of the insulin concentration in serum and of the FFA concentrations thus suggesting the presence of latent diabetes mellitus.

According to Sivers et al (33) and Wahlberg (42) the prognosis in cases of ischaemic heart disease seemed to be less favourable in patients with reduced glucose tolerance than in those in whom the tolerance remained normal. It might be of value to study whether the prognosis in cases of latent diabetes mellitus might become more favourable if the glucose tolerance were normalized by daily medication with for instance tolbutamide.

ACKNOWLEDGEMENT

This study was supported by grants from Consul and Mrs Ehrenfred Ovesén's Foundation.

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VOLUME CHANGES OF THE CALF DURING TEN MINUTES VENOUS STASIS

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Abstract Volume changes were examined in ten normal young experimental subjects by measurements on the calf with an air filled rubber cuff plethysmograph during venous stasis. Part of the experiment was performed while breathing 10% oxygen in nitrogen. The interpretation of the volume changes is discussed and it is suggested that from the fourth minute they are an expression of capillary filtration rate. After the initial rise which is due to arterial blood flow there is a transition phase where the volume increase is due to both venous filling and capillary filtration. Ten minutes breathing of 10% oxygen in nitrogen gave no changes in capillary filtration rate.

The capillary filtration rate (CFR) in the extremities can be measured by plethysmography. Krogh et al. (3) for example have used this method to determine the CFR in man. The principle of the measurement is that the increase in volume of an extremity or of a part of an extremity is measured during venous stasis, the assumption being that when the initial increase in volume ceases the elevated venous pressure during the stasis will cause an increased capillary pressure resulting in a rise in the filtration from the capillaries.

The present study aims at a closer analysis of filtration processes in the calf of the leg as well as at a description of the hemodynamic conditions which determine the processes of filtration in the extremities.

METHOD

An air filled rubber cuff plethysmograph (4) was placed on the thickest part of the calf. Venous occlusion was produced by means of a blood pressure cuff just proximal to the patella. The changes in volume were evaluated by changes in pressure in the plethysmograph recorded with a capacitance manometer. The signal was amplified and passed to a linear recorder.

The effect of venous occlusion lasting for 10 min was recorded.

Part of the experiment was performed while breathing 10% oxygen in nitrogen from a Douglas bag which was continuously filled with this gas mixture from a gas tank.

Experimental procedure

After recumbent rest for 30 min, the experimental subjects were examined on their left calf by the plethysmograph. The venous occlusion was produced on the thigh by pressures of 40, 60 and 70 mm Hg for periods of 10 min while breathing atmospheric air with at least 5 min pauses between the different pressures. The recording was repeated at a venous occlusion pressure of 60 mm Hg while breathing 10% oxygen in nitrogen. The experimental subjects had been breathing the gas mixture for 10 min before the measurement.

The room temperature varied between $\pm 6^\circ\text{C}$ but was constant during the individual measurement.

MATERIAL

The studies were performed with ten experimental subjects (6 men and 4 women) with a mean age of 23 years (from 20 years to 31 years).

RESULTS

Fig. 1 shows a plethysmograph curve during and after a 10 minute period of stasis. The figure is drawn on the basis of the mean values from the experiments at 60 mm Hg while breathing atmospheric air. It is seen that the curve contains three different phases. The initial slope in the plethysmograph curve expresses the resting blood flow. The second phase of the curve is seen in the 2nd minute (II) and the third goes from the 4th to the 10th minute. On releasing the occlusive pressure an immediate decrease in the volume is observed followed by a horizontal level for 5-10 seconds. The volume then slowly decreases. The final level not shown in the figure is reached after 2-3 min.

Table 1 shows the mean values of the plethys-

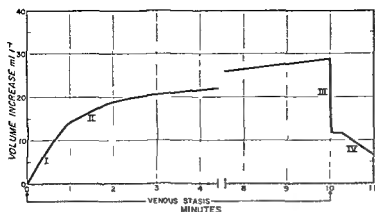


Fig 1 Plethysmograph curve during and after a 10 minute period of stasis

mographic measurements while breathing atmospheric air. The resting blood flow/volume was very similar (15.7 , 14.3 and 17.2 $\text{ml min}^{-1} \text{l}^{-1}$) at 40 , 60 and 70 mm Hg venous stasis. All other values in the table increase with increasing cuff pressure.

Table II shows the results of plethysmography with a venous stasis pressure of 60 mm Hg while breathing 10% oxygen in nitrogen and atmospheric air respectively. As a result of the reduction in the oxygen content in the inspired air

there is a significant rise in resting blood flow/volume of 6.5 $\text{ml min}^{-1} \text{l}^{-1}$ ($0.005 > P$).

There is also a significant rise in the volume increase in the 2nd minute of 1.8 $\text{ml min}^{-1} \text{l}^{-1}$ ($0.01 > P$). The change in the volume increase from the 4th to the 10th minute is not significant ($P > 0.05$).

The values for the immediate decrease in volume and the subsequent gradual decrease in volume after release of stasis (III and IV in Fig 1) were calculated but are not presented in the tables. The

Table I Mean values of the Plethysmographic measurements while breathing atmospheric air

Cuff pressure (mm Hg)		Resting blood flow/volume ($\text{ml min}^{-1} \text{l}^{-1}$)	Volume increase in the 2nd min ($\text{ml min}^{-1} \text{l}^{-1}$)	Capillary filtration rate ($\text{ml min}^{-1} \text{l}^{-1}$)
40	\bar{x}	15.7	3.4	0.63
$n=10$	S.D.	9.4	2.2	0.22
60	\bar{x}	14.3	5.0	1.12
$n=10$	S.D.	6.7	1.8	0.34
70	\bar{x}	17.2	6.7	1.37
$n=10$	S.D.	7.2	2.4	0.53

Table II Mean values of the plethysmographic measurement with a venous stasis pressure of 60 mm Hg and breathing respectively atmospheric air and 10% oxygen in nitrogen

Cuff pressure (60 mm Hg)		Resting blood flow/volume ($\text{ml min}^{-1} \text{l}^{-1}$)	Volume increase in the 2nd min ($\text{ml min}^{-1} \text{l}^{-1}$)	Capillary filtration rate ($\text{ml min}^{-1} \text{l}^{-1}$)
Atmospheric air	\bar{x}	14.3	5.0	1.12
$n=10$	S.D.	6.7	1.8	0.34
10% oxygen in nitrogen	\bar{x}	20.8	6.8	0.90
$n=10$	S.D.	9.1	3.2	0.50
Difference		+6.5	+1.8	-0.22
P		$0.005 > P > 0.001$	$0.01 > P > 0.005$	$0.10 > P > 0.05$

results showed that total volume increase immediate volume decrease and slow volume decrease all increased with increasing cuff pressure. There was no significant difference in these values as a result of breathing 10% oxygen.

DISCUSSION

When venous pressure is applied to an extremity a linear increase in volume will be observed during the first 10–20 seconds as an expression of the arterial blood flow as the venous return is stopped while the arterial flow continues. The pressure on the venous side will rise gradually and venous return under the occlusion cuff becomes reestablished. The question is when this state is reached in the human calf.

Frey (5) showed that venous return starts after 20 seconds in the hand. By simultaneous plethysmographic measurement on the hand and determination of venous pressure distal to the stasis cuff Frey showed that venous pressure rises to a constant value in 20 seconds while the volume increase continues although at a reduced rate. It has further been shown that an increase in femoral venous pressure of 10 mm Hg in the isolated dog hind limb resulted in stabilized peripheral venous pressure (digital vein) in less than 30 seconds (3). These studies included measurements of femoral venous pressure, peripheral venous pressure in a digital vein, arterial pressure, leg weight and venous outflow in the isolated dog hind limb. Following elevation of outflow pressure there was an immediate increase in all pressures and a rapid increase in weight (volume) associated with a pronounced transient decrease in venous outflow below the constant arterial inflow. These abrupt changes were followed by a leveling off of all pressures at values above control (the magnitude of change being dependent on the increment in the outflow pressure) and a slow steady gain in limb weight (volume = capillary filtration) which was associated with a venous outflow value slightly below the value for arterial inflow. When venous outflow pressure was lowered exactly analogous changes occurred in the opposite direction.

As shown in Fig. 1 a volume curve containing three different phases is obtained by plethysmographic measurement on the calf during venous stasis. After three minutes a slow continuous rise

is seen. However, when recording more distally on the extremity volume curves containing only two different phases were obtained: the second phase starting after the first minute. These types of curves were obtained by Celander (2) on the foot of newborn infants and by Nagasaka (9) on the fingers of adults. These differences are explained by the different contribution of skin and muscle at the various levels of the extremities.

The slope of the curve during the 2nd minute (II in Fig. 1) which indicates a volume increase could be explained by a gradual dilatation of the veins or in other words a pooling of the blood on the venous side exclusively, indicating that the venous pressure during the 2nd minute has not reached the tissue pressure caused by the stasis cuff. However, a venous outflow exists already after one minute. This may be demonstrated by further raising the venous occlusion pressure during a period of stasis. Thus it is indirectly shown that venous pressure after one minute is equal to the tissue pressure obtained from the stasis cuff. Owing to the valves in the communicating veins it is possible to have different pressures in the superficial and profound venous system. Measuring the venous pressure distally in a superficial vein would only give information on the pressure in the veins between the proximal and distal valves. The pressure in the profound venous system might be higher.

The slope in the 2nd minute in Fig. 1 therefore expresses venous filling, especially of the superficial venous system, although capillary filtration must contribute. On account of the valves in the communicating veins an outflow from the profound venous system may well exist while this is not possible for the superficial venous system.

As a result of the filtration in the muscle the pressure in the muscle tissue rises so that there is a counterpressure to the capillary pressure resulting from the venous stasis. Filtration will therefore again decrease as shown by Jacobsen et al. (7) and when there is no longer possibility of further increase in volume within the muscle fascia a steady state develops between fluid transport from the capillaries to the tissues and transport back from the tissues to the capillaries. The present investigation does not elucidate when this equilibrium is reached.

It may be assumed that the cutaneous filtration does not meet with any essential tissue

counterpressure during the period of measurement. The cutaneous filtration will therefore continue unimpeded. This is in accordance with clinical experience that edema of the skin may be monstrous while edema of the muscle is always moderate.

Consequently the volume increase during the 2nd minute cannot be caused by capillary filtration alone as suggested in a preliminary report (10). In previous studies (10) it was shown that moderate acute carbon monoxide exposure significantly augmented the volume increase during the 2nd minute. Further experiments have confirmed this observation. Breathing 100% oxygen or 15% oxygen in nitrogen did not change the slope while breathing 10% oxygen in nitrogen significantly increased the slope in phase II as is also shown in Table II. It is impossible at present to explain this observation.

Asmussen et al. (1) applied an air-filled plethysmograph on the calf and used the volume increase from the 2nd to the 7th minute as an expression of capillary filtration. In the present work the volume increase from the 4th minute has been used to indicate capillary filtration.

Experimental confirmation of the theoretical considerations for using a plethysmograph to measure capillary filtration rate has been established for example by Gayton et al. (6). They measured fluid movement through the capillary membrane in the lower legs of dogs by using implanted perforated capsules as internal plethysmographs. By this technique it was possible to compare a decrease in the interstitial fluid pressure with the effects of changes in venous pressure and arterial pressure. Their results confirmed Starling's hypothesis that changes in interstitial fluid pressure can affect movement of fluid through the capillary membrane in the same way as do changes in capillary pressure, plasma colloid osmotic pressure and interstitial fluid colloid osmotic pressure.

If the stasis is released after the lapse of ten minutes the volume increase will be reduced at two different rates: a rapid phase which must be due to the displacement of the intravascular volume and a slow phase determined by back diffusion of the fluid to the capillaries and possibly also transport through the lymphatic vessels. The horizontal level of about 10 seconds duration at the transition between the two phases sug-

gests that a certain time is taken to refill the capillary network which has become emptied as a result of the increased tissue pressure.

Table II shows that exposure to 10% oxygen in nitrogen gave no increase in capillary filtration rate in the leg. An increase was to be anticipated since high altitude experiments (11) have shown a hemoconcentration and a decrease in plasma volume indicating an increased capillary filtration rate. However, the present experiment only exposed the subjects to 10% oxygen in nitrogen for ten minutes before the measurements. This exposure seems to be of too short duration to provoke any effect on the capillary filtration.

In calculating the capillary filtration coefficient (CFR/mm Hg) Celerander (2) assumes that a rise in the venous stasis pressure causes a rise of 80% in capillary pressure. For this reason the difference between volume increases at 60 mm and 40 mm Hg stasis pressure has been divided by 16 instead of 20 in order to obtain an expression of volume increase per mm Hg. Volume increase from the fourth to the tenth minute per mm Hg thus becomes $0.051 \text{ ml min}^{-1} \text{ l}^{-1} \text{ mm Hg}^{-1}$. This is of the same order of magnitude as the CFR/mm Hg obtained by other authors.

ACKNOWLEDGEMENTS

This work has been aided by grants from the Danish National Association against Rheumatic Diseases and Arvid Nilsson's fund.

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SERUM AND URINARY URIC ACID IN RESPIRATORY ACIDOSIS

PRELIMINARY REPORT

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Abstract Serum and urinary uric acid were measured in seven patients suffering from severe respiratory acidosis. The highly elevated serum levels of uric acid decreased to normal after carbon dioxide had been blown off with assisted ventilation. Simultaneously the urinary excretion of uric acid increased.

The development of secondary hyperuricaemia may be due to increased uric acid formation or decreased elimination. Elimination is principally achieved through excretion by the kidneys. Although this mechanism is not fully understood, increasing evidence supports the current view that uric acid after glomerular filtration is largely reabsorbed and later actively secreted in distal tubuli (17). Agents which increase tubular resorption or impair tubular secretion may cause hyperuricaemia.

It has been shown that the infusion of lactic acid considerably decreases renal uric acid elimination in normal healthy men without a change in the inulin or PAH clearances (18). In several other clinical conditions known to be associated with increased lactic acid levels, the serum uric acid has been shown to be elevated in toxæmia of pregnancy (6), after physical effort (19), after intake of alcoholic beverages (7), in arterio-sclerosis (3, 10), in patients with von Gierke's disease (4), etc. If blood lactate is elevated, the decreased excretion of urate leads to hyperuricaemia.

A similar effect can be achieved with ketone bodies such as beta-hydroxybutyrate or acetoacetate. It has also been shown that a high fat diet and starvation lead to diminished uric acid elimination, resulting in hyperuricaemia (12).

Against this background it was interesting to

study the influence of respiratory acidosis on serum uric acid levels and urate excretion in a small number of patients treated for respiratory insufficiency in the intensive therapy unit of the Department of Medicine, University of Oulu.

MATERIAL AND METHODS

The material consisted of seven consecutive patients admitted to the intensive therapy unit for respiratory acidosis. Their clinical condition had deteriorated progressively over several days to such an extent that intensive therapy was necessary. In three patients with chronic bronchitis and pulmonary emphysema, the cause of severe deterioration was pulmonary infection. One patient with generalized obstructive airway disease developed acute spontaneous pneumothorax, which was the final cause of deterioration. In three patients with bronchial asthma, intense and prolonged obstruction led to severe alveolar hypoventilation and to clinical symptoms of CO retention.

The series according to age, sex and diagnosis is presented in Table I.

The patients were treated according to currently accepted principles. They received antibiotics, bronchodilating agents, expectorants, corticosteroids and digitalis preparations if necessary. Initially all patients received fluids parenterally and thereafter orally since adequate hydration

Table I. Distribution of seven patients admitted to the intensive therapy unit for respiratory acidosis by sex, age and diagnosis

Patient	Age	Sex	Diagnosis
K. M.	62	♀	Chronic bronchitis with emphysema
M. P.	18	♂	Bronchial asthma
M. J.	67	♂	Chronic bronchitis with emphysema
H. H.	63	♀	Bronchial asthma
G. A.	54	♀	Bronchial asthma
M. V.	51	♂	Spontaneous pneumothorax
P. J.	67	♂	Chronic bronchitis with emphysema

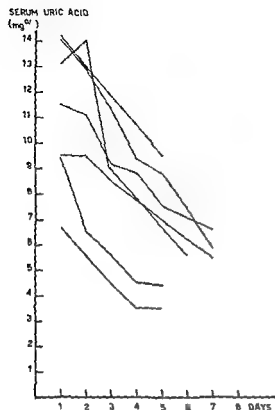


Fig. 1 Serum uric acid (mg/dl) under treatment of respiratory acidosis

helps to liquefy the secretions and expedites their removal. A locally humid atmosphere was also used to facilitate the loosening and expectoration of secretions. In three cases the secretions were removed by aspiration through tracheostomy. Use of intermittent positive pressure breathing also promoted bronchial drainage and further assisted the removal of secretions. In all the cases intermittent positive pressure breathing (the Bennet Model PR 1 A or PR A) with 40% oxygen was used to blow off carbon dioxide. The Astrup values using the capillary method (2) were analysed daily (or several times a day). The serum uric acid was measured daily in each patient as was the amount of uric acid excreted. The measurements of uric acid were performed using the colorimetric Auto-Analyzer method (6). Facilities for determination of lactic acid were not available in this study. The period of treatment and observation of patients in the intensive therapy unit varied from three to seven days.

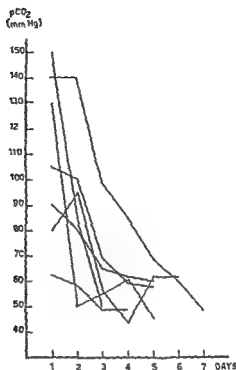


Fig. 2 $p\text{CO}_2$ (mm Hg) measured daily under treatment of respiratory acidosis

RESULTS

It can be seen from Figs. 1 and 2 that improved alveolar ventilation leads to a progressive decrease in the high $p\text{CO}_2$ values during treatment and high serum uric acid levels show a similar decline.

Table II shows daily mean values of serum uric acid, $p\text{CO}_2$, blood pH and urinary uric acid of the patients.

During the first day while the excretion of uric acid is still fairly low and serum uric acid high, the blowing off of carbon dioxide and better oxygenation achieved by assisted ventilation and other treatment caused a considerable increase of urinary uric acid excretion and a progressive decrease in serum uric acid.

Table II The mean values of serum uric acid, urinary uric acid, blood pH and blood $p\text{CO}_2$ in seven patients with respiratory acidosis during intensive therapy

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Serum uric acid (mg/dl)	11.2	10.4	8.3	7.3	6.8	5.7	5.9
Urinary uric acid (mg/24 h)	351	573	660	580	415		
pH	7.223	7.259	7.342	7.422	7.378		
$p\text{CO}_2$ (mm Hg)	106	101	64	60	57		

DISCUSSION

Evidently respiratory acidosis leads to considerable hyperuricaemia though this finding has not been reported earlier in the literature. All the present patients had hyperuricaemia of considerable magnitude prior to the initiation of intensive therapy for respiratory acidosis. It appears that urinary elimination of uric acid is impaired under respiratory acidosis.

Although in this series large quantities of uric acid were excreted even on the first day this was only an early result of effective therapy. The present results show that the amounts excreted almost doubled as respiratory acidosis decreased. Although hyperuricaemia in these patients cannot be attributed to diminished excretion alone excretion increased remarkably as carbon dioxide and simultaneously the serum uric acid fell to normal levels.

The results and findings reported earlier in the literature support the view that acidosis itself regardless of its origin leads to hyperuricaemia. It has been shown for example that infusion of fructose produces an elevation of serum lactic acid and serum uric acid. It also increases the urinary urate excretion (9). Similar increased uric acid formation has been shown to exist in one patient suffering from von Gierke's disease (1). In von Gierke's disease hyperuricaemia coexists with an elevated serum lactic acid and a decreased renal urate clearance (5).

The intake of alcoholic beverages results in lactic acidemia and hyperuricaemia. The small decrease of urinary urate excretion is insufficient to explain elevated serum values (8). Sorensen (15) has shown that uric acid can also be excreted into the gut. In patients with renal insufficiency up to 70% of the uric acid is eliminated in this way (15). The influence of respiratory acidosis on this intestinal route of elimination has not been studied nor is it mentioned in the literature. All the present patients had normal renal function and daily urinary volumes. An essential cause of hyperuricaemia induced by respiratory acidosis is decreased urinary uric acid excretion. This theory is also supported by the decreased renal urate clearances recorded in connection with infusions of lactic acid or ketone bodies. However the possibility of increased uric acid formation as an essential factor in these conditions has not been absolutely ruled out (9).

Uric acid ultimately consists of xanthine and hypoxanthine influenced by xanthine oxydase enzyme. Seegmüller et al (13) described a syndrome of hyperuricaemia accompanying a congenital defect of the guanine hypoxanthine phosphoribosyltransferase enzyme. Uric acid formation is greatly increased in these patients although the exact mechanism of the increase is not fully understood. Thus these enzymes could be influenced by acidosis so as to increase the production of uric acid.

In patients with myocardial infarction and with cerebrovascular damage (3, 14) the hyperuricaemia may be largely due to coexistence of metabolic acidosis known to occur under these conditions. However it has been shown that in patients with myocardial infarction the serum uric acid remains at an elevated level for a considerable period of time even after the acidosis has disappeared (14).

In secondary hyperuricaemia clinical manifestations may also occur in the form of acute gouty arthritis in connection with sudden changes of serum uric acid levels (8) both in haemodialysis and starvation. A patient unconnected with the present series who had severe respiratory acidosis and high serum uric acid contracted an acute attack of gouty arthritis on the sixth day of treatment. His serum uric acid had fallen from 11.6 to 5.7 mg%. The typical attack involved the metatarsophalangeal joint of the big toe. Even in secondary hyperuricaemia produced by respiratory acidosis it remains obscure why an acute attack of arthritis should manifest itself occasionally when the uric acid levels are remarkably low and why in other cases no clinical symptoms occur despite greatly elevated serum levels.

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APPARENT RESISTANCE TO ORAL ANTICOAGULANT THERAPY AND INFLUENCE OF HYPNOTICS ON SOME COAGULATION FACTORS

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Abstract The influence on the one-stage prothrombin time (Quick index) prothrombin + proconvertin activity platelet count and fibrinogen level of a barbiturate Diminal duplex[®] (Astra) (10 patients) and of a nonbarbituric hypnotic drug Placidyl[®] (Abbott) (6 patients) was studied during treatment with bishydrocoumarin AP[®] (Ferrostan). An increase in the Quick index was noted in both groups whereas the prothrombin + proconvertin activity fibrinogen level and platelet count were unaffected. The dose response of bishydrocoumarin on Quick index was studied in 10 patients. Two of these did not respond to large doses of bishydrocoumarin. Other anticoagulants such as Waran[®] Sintroma[®] and Tromexan[®] were also ineffective when given orally. Intravenous warfarin (Waran[®]) effectively lowered the Quick index in one of the patients but not the other. One patient resistant to bishydrocoumarin successfully treated with Marcoumar orally is also described. Apparent resistance to oral anticoagulants during treatment with hypnotic drugs and different causes of anticoagulant resistance per se are discussed.

During treatment with coumarin derivatives the patients are often given other drugs. A decreased prothrombin response after administration of barbitol compounds has been reported (2, 8, 9, 21). The purpose of the present investigation was to study the effect of certain drugs on blood coagulation estimated by platelet count prothrombin + proconvertin activity one-stage prothrombin time (Quick index) and fibrinogen level.

MATERIAL AND METHODS

Patients being given anticoagulant therapy (bishydrocoumarin AP[®] Ferrostan Sweden) were studied; they were free from known liver kidney or gastrointestinal disease and were fed on a regular hospital diet. Only those patients were included who after at least one week of coumarin treatment had a stabilized therapeutic Quick index or prothrombin + proconvertin (P+P) activity. No hypnotics were given for a week before the investigation. Bishydrocoumarin and the relevant drug were administered at 8 p.m. Blood specimens were drawn every day at 8 a.m.

A fixed dose of bishydrocoumarin was given during the 23-day experimental period. The influence was studied of two hypnotics—i.e. Diminal duplex[®] (vinbarbital sodium 100 mg and aprobarbital 50 mg Astra Sweden) and Placidyl[®] (3-(2-chlorovinyl)penten-(1)-ol Abbott)—on the platelet count prothrombin + proconvertin activity one-stage prothrombin time (Quick index) and fibrinogen level. Other apparent resistance to treatment with coumarin derivatives was also noted.

The one-stage prothrombin time (Quick index) was determined as described by Lehman (12). The platelet count was determined according to Christensson (11) and prothrombin + proconvertin activity according to Öwren and Åas (20). Fibrinogen was determined by the method of Bergström et al. (3).

RESULTS

Ten patients with deep venous thrombosis and a Quick index of about 30° were given two tablets of Diminal duplex[®] daily. The Quick index and prothrombin + proconvertin activity were recorded. After 18 days administration of the hypnotic the Quick index rose from an average 36° to 53° (Fig. 1). Although the prothrombin + proconvertin activity rose in six patients no significant changes occurred. No consistent changes were noted in platelet count and fibrinogen level. When hypnotic therapy was discontinued the Quick index slowly returned to the normal level within about a week. Thus barbiturate administration was associated with an increase of the Quick index.

After six patients with myocardial infarction had been treated for two weeks with bishydrocoumarin they were given concurrently a daily dose (1 g) of Placidyl[®] as a hypnotic. During the subsequent 18 days the Quick index rose from an average 38.6 to 55° (Fig. 2). No significant change took place in the prothrombin + proconvertin activity fibrinogen level or platelet count.

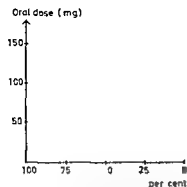


Fig 1 Oral dose of bishydrocoumarin (mg) and Quick index (°) before (●) and after (○) concurrent administration of a barbiturate (Diminal duplex® Astra)

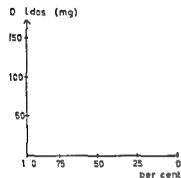


Fig 2 Oral dose of bishydrocoumarin (mg) and Quick index (°) after two weeks therapy (●) and after 18 days concurrent administration of 1 g of Placidyl® (Abbott) daily (○)

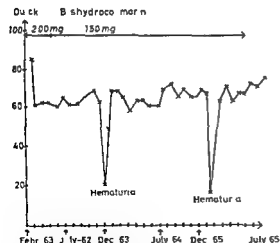


Fig 3 Quick index (°) in a 6-year-old man (O B) treated after a myocardial infarction with bishydrocoumarin and a regular evening dose of Placidyl® (1 g)

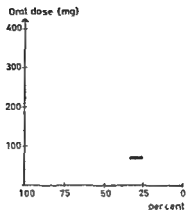


Fig 4 Quick index (°) in 20 patients treated with bishydrocoumarin

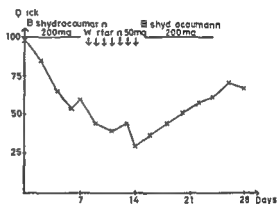


Fig 5 Resistance to oral and intravenous anticoagulant therapy

Resistance to coumarin therapy was noted in one patient. He was a 62 year old man (O B) who following a myocardial infarction was treated with bishydrocoumarin (AP®) and regularly took Placidyl® (1 g) every evening as a hypnotic. The dose of anticoagulant and his Quick index are shown in Fig 3. Two episodes of haematuria occurred, his Quick index—which was normally high despite large doses of AP—fell concurrently to 21° and 14°. When the bleeding occurred he had been without Placidyl for 4 and 6 days respectively.

Fig 4 shows the relation between the Quick index and the oral dose of bishydrocoumarin in 20 patients. They were treated with the anticoagulant for one week and the same dose was given for seven days before starting the investigation. It is remarkable that two of these patients did not respond to large doses of bishydrocoumarin. One was a 68 year old woman (A E).

with a history of at least two pulmonary thromboembolisms. The other was a 61 year-old man (G O) with a deep thrombosis of the calf. The lowest Quick index recorded during the whole treatment period was 52° in the woman and 58° in the man. In these two patients other coumarin derivatives such as warfarin (Waran®) (Nyegaard), Sintroma® and Tromexan® (Geigy) were also ineffective when given orally. Intra-venous injection of 50 mg of Waran reduced the Quick index in the man (Fig 5) but had no effect in the woman (Fig 6).

Fig 7 shows resistance to bishydro coumarin appearing in a 58 year old woman (S A) with a myocardial infarction. A change to Marcoumar® (Roche) successfully lowered her Quick index.

DISCUSSION

Avellaneda (2) and Grilli (7) observed a decrease in the prothrombin time after addition of various barbiturates in patients treated with ethyl biscoumaracetate. Reverchon and Sapir (21) found that the dose of this anticoagulant had to be increased when barbiturates—of which they often prescribed extremely large doses—were given concurrently. The present study shows that administration of a barbiturate and of Placidyl® in combination with another dicoumarol compound—bishydrocoumarin (AP®)—seemed to increase the Quick index but did not change the P+P activity. The same observation on the P+P level after barbiturate intake was made by Samuelsson and Lilienberg (22).

The coumarin anticoagulants influence the clotting mechanisms in the same way by interfering in the synthesis of clotting factors II, VII, IX and X. Since barbiturates influence the Quick index

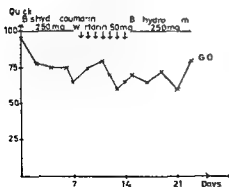


Fig 6 Resistance to oral anticoagulant therapy

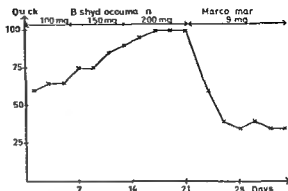


Fig 7 Quick index (°) in a 58 year-old woman (S A) with myocardial infarction during treatment with bishydrocoumarin and Marcoumar. Resistance to the former appeared during treatment.

but not the prothrombin + proconvertin activity, fibrinogen level or platelets, it is possible that they could influence factor X.

The mechanism of barbiturate action on the prothrombin complex is unknown. Enzyme stimulation was suggested by Weiner (25) and Cucinell et al (5). An increased activity of liver enzymes which metabolize dicoumarol has in fact been noted in rats after phenobarbital treatment. Dayton et al (6) showed that pretreatment with phenobarbital lowered the plasma drug level of dicoumarol and diminished the decrease in the prothrombin response when dicoumarol was given orally but not when administered intravenously. Aggeler and O'Reilly (1) demonstrated that barbiturate decreased the absorption of dicoumarol from the gut to the blood and that greater amounts of the drug could be detected in the stools. They also noted that the barbiturate effect on the dicoumarol persisted for some weeks after withdrawal of the hypnotic.

When such drugs are administered, larger doses of the oral anticoagulant seem to be necessary.

Apparent resistance to the hypoprothrombinemic effect of therapy with coumarin derivatives has been reported in man and in animals (17, 24) both as an acquired phenomenon and as an inherited trait (19). Hyperlipaemia (10), gastrointestinal disorders (23), pulmonary infarction (18), lactation (13) and concomitant administration of other drugs (13) may also produce such resistance to coumarin therapy.

The present study supports the concept of different mechanisms of coumarin resistance.

The findings of resistance to oral but not to intravenous therapy (Fig 5) are in line with the view that barbiturates decrease the absorption of coumarin derivatives presumably due to influence on an active absorption mechanism

Spontaneous resistance to dicoumarol and warfarin occurred in this material as described earlier. One possible explanation is an extremely rapid metabolism earlier demonstrated by O'Reilly et al (19)

In investigated cases the Quick index measuring the coagulation factors II VII IX and X was increased whereas the prothrombin + proconvertin actively measuring only the factors II and VII was unchanged. The inference is that in contrast to earlier explanations the barbiturates (and placidyl) do not influence the metabolism of dicoumarol derivatives but their effect on the factors IX and X of the coagulation system

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COBALT INDUCED HYPOTHYROIDISM AND POLYCYTHEMIA IN LIPOID NEPHROSIS

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Abstract An 11 year-old boy who had been suffering from genuine lipoid nephrosis from the age of 6 / years received thiosemicarbazone for three weeks. During this therapy severe anemia developed. The anemia was treated with cobaltous chloride. During the fourth month of therapy the child developed very severe hypothyroidism. There was considerable enlargement of the thyroid gland along with moderate polycythemia. These events are thought to be due to the cobalt therapy.

In 1929 Waltner and Waltner (48) observed that administration of cobalt salts to rats produced polycythemia. This erythropoietic effect of cobalt salts has been repeatedly confirmed. Consequently cobalt has been used for the treatment of various refractory types of anemia including the nephrogenic form (3, 13, 22). Massino and Pantarotto (29) reported a marked increase in erythropoietin concentration in the blood of azotemic patients during cobalt treatment. This increase in erythropoietin was accompanied by an improvement in the anemia.

Genuine lipoid nephrosis resistant to treatment with corticosteroids is still a therapeutic problem. Heilmeyer (17) originally suggested that thiosemicarbazone (TSC) could be used in the treatment of the nephrotic syndrome and since then it has been used in this disease by many authors.

In this paper a case of genuine lipoid nephrosis in a male child will be presented. During TSC therapy severe anemia developed and this was treated with iron and cobaltous chloride. In the course of this treatment polycythemia, goitre and hypothyroidism developed.

CASE REPORT

The patient was a 1 year-old boy whose brother had suffered from an edematous renal disease from which, however, he had recovered. No thyroid diseases had oc-

curred in the family. The patient was in good health until the age of 6 years (in 1961) when he had an acute infection followed by typical signs of lipoid nephrosis. He was initially treated with corticotrophin (ACTH) and corticosteroids in a local hospital but as there was no improvement in his condition he was transferred to the Children's Hospital, University of Helsinki. At that time he had abundant proteinuria (ad 14 %) electrophoresis of the serum proteins revealed a decrease in albumin and gamma globulin and an increase in alpha₂ globulin. The serum cholesterol was elevated the highest value being 1030 mg/100 ml. The ESR was 135 mm/h and the BP 150/100 mm Hg. He was again initially treated with ACTH and later with prednisone intermittently. Attempts were made to increase the urine excretion with diuretics but only spironolactone was effective. During this treatment there was clinical improvement and a concomitant improvement in the laboratory tests. After discharge from hospital treatment was continued with ACTH, prednisone and diuretics. For the next two years the patient was followed up at the outpatient department and was temporarily hospitalized a few times. The nephrotic signs, however, persisted in varying degree.

In April 1963 a Cu-hingoid syndrome developed and prednisone treatment was stopped.

In October 1963 he was again hospitalized since the edema had increased. After a short course of prednisone the treatment was continued with chlorbutalolone alone. His condition deteriorated but treatment with corticosteroids was not started because the patient had been exposed to chickenpox. Instead TSC treatment was introduced (75 mg three times a day). After two weeks of treatment the patient became tired, complained of nausea and began to vomit. His Hb had decreased from 12.8 to 7.5 g/100 ml. X-ray pictures of the long bones showed changes compatible with leukemic infiltrations. The white blood picture, however, was normal. The bone marrow smear indicated the possibility of hemolytic (uremic) anemia and since the blood urea was elevated (63 mg/100 ml) this explanation seemed to be most likely. The patient was given blood transfusions and treatment with cobaltous chloride and iron was introduced. The daily dose of CoCl₂ was 10 mg and of iron 45 mg. At the same time the patient got rumps, which ran an uneventful course. He was discharged and treated at home with anabolic steroids and cobalt iron.



Fig 1 The patient after four months of cobalt therapy. Note the enlargement of the thyroid gland.

In March 1964, after three months of this treatment, he became increasingly tired and complained of pain in the chest, dyspnea occurring even after moderate exercise and a feeling of compression in the neck. He became slow and somnolent and his skin was dry and coarse. On physical examination the reflexes were sluggish and the thyroid, previously normal in size, was now estimated by palpation to be about 100 g (Fig 1). There was no tenderness. The blood picture showed marked polycythemia (Hb 18.6 g/100 ml, RBC 5.78 mill/mm³). Bone marrow biopsy revealed no abnormalities. The PBI was repeatedly low (1.3 to 1.8 gamma/100 ml) and the BEI was 0.4 gamma/100 ml. The serum cholesterol was 150 mg/100 ml. No thyroid antibodies (hemagglutination and complement fixation tests) were present. No LE cells were found and the antinuclear factor was negative. Anti-

strepto- and antistaphylococcal titers were normal. The ECG showed low voltage amplitudes. Because of the very low cardiac output, the pulse rate was however relatively high. After treatment with thyroxine, there was rapid change in the voltage and pulse rate (Fig 2). Intravenous pyelography revealed slow excretion of dye but no evidence of renal tumour. Histological examination of a specimen of thyroid tissue, taken surgically, showed an extremely activated histological picture. The epithelium was very high and most follicles were devoid of colloid. There were no signs of malignancy or of giant-cells or lymphoid thyroiditis (Fig 3). Although cobalt treatment was discontinued immediately, the patient had severe spells of tiredness and fatigue during which the BP decreased to 95/90. For this reason, radioactive iodine tests could not be carried out before starting treatment with thyroxine.

Treatment with thyroxine 55 µg/day was begun and within nine days the patient's condition had improved considerably, whereupon the thyroxine dose was increased to 110 µg a day. After three weeks treatment, the serum cholesterol decreased to 640 mg/100 ml and the thyroid returned to normal size. Throughout this period, the nephrotic syndrome was treated with diuretics only.

The patient was discharged in May 1964 in fairly good condition. On repeated follow-up examinations since then, he has been doing well on thyroxine and diuretics. The PBI has varied from 3.1 to 6.8 gamma/100 ml, the serum cholesterol from 398 to 844 mg/100 ml, the blood urea from 19 to 37 mg/100 ml and the proteinuria from 0.17 to 0.00 g/day. The size of the thyroid has remained normal.

DISCUSSION

It was primarily assumed that the patient presented in this paper had anemia of renal origin. Leakage of transferrin into the urine has been suggested (6) to cause iron deficiency in nephrogenic anemia, but the possibility of faulty synthesis of this carrier protein cannot be dismissed. Gitlin et al (14) found synthesis to be normal but

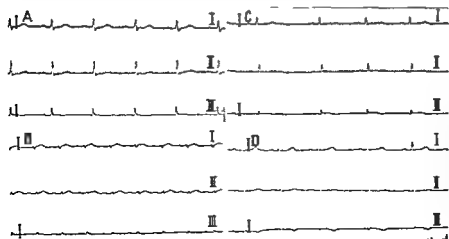


Fig 2 ECG (A) Before the hypothyroid state (B) During the hypothyroid period (C) Six days after introduction of thyroid therapy (D) Twelve days after introduction of thyroid therapy (E) 1 mV. Note the changes in the amplitude and pulse rate.

showed that nephrogenic anemia may be due to leakage and to increased catabolism of transferrin. Hence it is possible that in the present case iron stores were decreased.

Development of anemia has been observed during treatment with thiosemicarbazone (TSC) (41). Bone marrow changes have been non specific with out any qualitative alterations (41). Pribilla and Koester (34) reported six cases of hemolytic crises during TSC therapy.

It seems probable that the anemia in this case was produced both by the action of TSC and by the increased catabolism and leakage of transferrin.

That cobalt stimulates erythropoiesis is a well established fact but the exact mechanism is still obscure. It has been claimed that the hypoxia in bone marrow caused by cobalt releases red cells from the marrow (2) but other investigators (25) have been unable to confirm these findings. Cobalt is known to interfere with tissue respiration (9-46) and it is suggested that the hypoxia thus created stimulates erythropoietin production (4-10). Whether this is related to cytochrome oxidase activity (42) or to a renal erythropoietin inhibiting factor (11) is not known. It might be that there are other sites of erythropoietin production apart from the kidney (45).

Orten (30) suggested that the vasodilatation caused by cobalt results in local anoxia and it has later been shown (20) that the erythropoietin stimulating effect of cobalt may be related not only to histotoxic anoxia but also to stagnant hypoxia following a severe decrease in cardiac output.

During treatment with cobalt polycythemia developed in our patient. It seems likely that this was a result of the treatment with cobalt. The possibility that the anabolic steroid given during the same period as the cobalt contributed to the development of this condition is not ruled out. Polycythemia probably due to increased erythropoietin production has been described in various renal diseases (12) but the presence of anemia in our patient before the development of polycythemia argues against this possibility.

Cobalt has been reported to induce a variety of adverse reactions of which anorexia, nausea, vomiting and diarrhea are the most common (3-13). Others include erythema and hot sensations (3), skin rashes (51) and substernal aches (3).

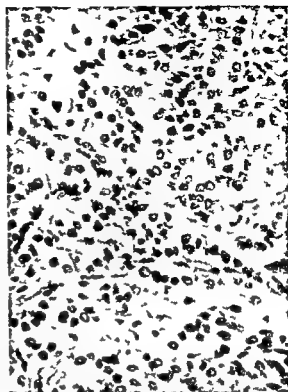


Fig. 3. Microscopic appearance of the thyroid gland. A surgical biopsy H&E, $\times 240$. Note the high epithelium and the practically total lack of colloid.

Further serious complications are tinnitus and neurogenic deafness (13).

The effect of cobalt salts on the thyroid was first reported by Gross et al. (16) who observed the development of goitre and hypothyroidism in children given cobaltous chloride for the treatment of anemia but the idea that cobalt might be a thyrostatic agent was initially challenged by other authors (19). Since then however cases have been reported in which goitre and hypothyroidism have developed during treatment with cobalt (7-49). Roche and Layrisse (39) observed a significant decrease in the thyroidal uptake of ^{131}I in euthyroid subjects after two weeks treatment with cobaltous chloride; the uptake was in fact almost totally blocked. Pimentel, Malaussena et al. (33) observed a therapeutic effect in hyperthyroid patients as well as decreased thyroidal ^{131}I uptake after treatment with cobalt. These effects have been confirmed by other authors (31). Thiocyanate has been reported to cause an outpouring of radioactive iodine during cobalt administration, indicat-

ing the presence of a binding defect in the thyroid (31) The goitrogenic effect of cobalt in man thus seems to have been amply demonstrated

The findings in experimental animals however have been more controversial In studies with guinea pigs Antila et al (1) observed the production of goitre histological activation of the thyroid and a decrease in PBI after cobalt administration whereas similar studies in rats mice rabbits and young chicks have produced unequivocal results In 1 day old chicks however Kriss et al (24) were able to produce a decrease in the thyroïdal uptake of ^{131}I and an increase in the uptake of ^3P but no significant colloid depletion TSH and propylthiouracil (PTU) induced very marked colloid depletion TSH is known to increase the thyroïdal uptake of ^3P in from 1 to 3 day old chicks (26) and to produce changes in the colloid epithelium ratio The same was observed in the study of Kriss et al (24) Administration of PTU to rats induces histological activation as well as an increase in the uptake of ^3P by the thyroid (47) Kriss et al (24) drew the conclusion that because of the discrepancy between the effect of cobalt on the uptake of ^3P and colloid depletion as compared to that of PTU the mode of action of cobalt on thyroïdal iodine metabolism was not similar to that of PTU It must be borne in mind however that the uptake of ^3P in the thyroid reflects general cellular activity and epithelial growth (27) Hence it stands to reason that whatever the precise mode of action of cobalt might be the ion must induce some kind of blocking of intrathyroïdal iodine metabolism which in turn and in the same manner as other goitrogenic substances leads to goitre through the mediation of TSH However this has not been further explored by newer methods of investigation Blocking of the protein binding of iodine seems to occur anyway

As regards the mode of action it has been observed by Levy et al (28) that the activity of some SH dependent enzymes such as succinoxidase and cholinoxidase as well as that of the non SH dependent cytochrome oxidase and catalase is inhibited by cobalt in an aerobic milieu This may have some bearing at least on the hematological effects of cobalt (43) but it is also known that other inhibitors of cytochrome oxidase such as cyanide azide sulphide and carbonmonoxide inhibit the formation of diiodothyrosine and thy-

roxine (40) Some studies of Kirkwood and Fawcett have been quoted by Jaimet and Thode (19) and Kriss et al (24) according to which cobalt in a 10^{-3}M concentration exerts an inhibiting effect on thyrosine iodination in a cell free system

It has been suggested that the effect of cobalt on the thyroid might be some kind of allergic reaction (7) Furthermore the idea has been advanced (49) that some kind of hereditary primary defect in thyroid iodine metabolism must exist before cobalt can exert its goitrogenic effect This is thought to hold good as far as iodine induced goitrous hypothyroidism is concerned and of course if such a defect is present any kind of goitrogen may produce evident thyroid manifestations no matter how small the dosage This however is still far from settled

Different results obtained by various workers may merely reflect difference in dosage in intestinal absorption in iodine supply etc In fact the intestinal absorption of cobalt especially if administered after a meal seems to vary greatly (32) Cobalt is rapidly absorbed and disappears rapidly from the blood which makes the blood concentrations vary widely Most cases of cobalt induced goitrous hypothyroidism have been reported in children The different rates of occurrence in children and adults may be due to different dosage schedules In adults one is used to a definite steady daily dosage and only two cases of goitrous hypothyroidism in adults have been reported (both with chronic renal disease) In children cobalt is administered according to body weight which might result in blood levels relatively higher than in adults It is conceivable that faulty renal excretion may be of importance In the case reported here the glomerular filtration rate was substantially decreased because of the primary disease and the TSC therapy (41)

The role of TSC in the development of goitre and hypothyroidism in this case is unknown The formula of TSC bears some resemblance to that of the thiocarbamides Corti and Romualdi (8) found histological changes in the thyroid of rats after TSC treatment resembling those found after thiocarbamide treatment but it has been reported only once (44) that TSC may have caused goitre In the present case the goitre appeared fairly rapidly after introduction of cobalt therapy but the additional affect of the preceding TSC treatment cannot be excluded

The hypercholesterolemia and the decreased basal metabolic rates originally suggested that the thyroid function in the nephrotic syndrome might be impaired. This in turn might have some bearing on the nephrosis itself. The PBI has also occasionally been found to be low. Thyroid therapy has been tried but without improvement of the clinical condition or the PBI (21). Holsti (18) described a hypothyroid patient in whom nephrosis developed and in this case thyroid treatment clearly improved the nephrotic condition. In the present case there was initially a certain degree of improvement after thyroxine treatment was introduced but it is quite possible that remission was only temporary. The low PBI found in nephrosis is thought to be due to a low level of binding sites in the thyroxine binding globulin (TBG) in the serum (38) resulting from leakage of this carrier protein into the urine (21, 35). There is usually no histological alteration in the thyroid gland in patients suffering from the nephrotic syndrome (36) although in one case change have been observed (50). There might, however, be a decrease of iodine stores in the thyroid since Recant and Riggs (36) found a lowered response to TSH in nephrotic patients after methimazole treatment. In the present case the PBI was low but determination of the TBG could not be carried out.

In this case serum cholesterol elevation as thought to be the result both of the nephrotic syndrome and of hypothyroidism. Cobalt on the other hand is known to have a lipogenic effect (5). This effect has been postulated to be due to a lipid mobilizing hormone (53) the concentration of which has been reported to be increased in the blood of patients with the nephrotic syndrome (52).

The patient described here had mumps immediately before the hypothyroidism occurred but thyroid biopsy and the absence of thyroid antibodies in the blood seem to exclude the possibility that subacute thyroiditis was the cause of the events.

We submit therefore that the hypothyroidism and goitre in this case were due to the administration of cobalt for treatment of the anemia produced by TSC.

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RELATION OF TUBULAR MAXIMUM REABSORPTION OF GLUCOSE AND PARATHYROID FUNCTION IN GOATS

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Abstract A well-defined tubular maximum reabsorption of glucose is demonstrated in goats. The reabsorptive capacity is not affected by spontaneous changes of the glomerular filtration rate. Administration of EDTA or parathyroid extract led to increased tubular reabsorptive capacity for glucose while calcium administration or parathyroidectomy had the opposite effect. These observations support the previous assumption of a positive relationship between the tubular reabsorptive capacity for glucose and the parathyroid function.

Recent observations in humans indicate a positive correlation between tubular reabsorptive capacity for glucose and parathyroid function (1, 2). Increased values of the ratio TmG/GFR (that is tubular maximum for glucose related to the glomerular filtration rate) were found in patients with hyperparathyroidism and decreased values in patients with hypoparathyroidism compared to the values obtained in patients with no signs of endocrine disorders.

The purpose of this report is to demonstrate a well-defined tubular maximum reabsorption of glucose in goats and to relate changes of TmG to induced alterations of the parathyroid function.

MATERIAL AND METHODS

Four young female goats weighing 24 to 43 kg. were used for the investigation. In three goats (A, B and C) a glucose titration study that is determination of tubular reabsorption of glucose under increasing filtered load of glucose was performed to decide whether or not the goat has a well-defined TmG . TmG was determined twice at intervals of 4 to 8 days in three goats (B, C and D) directly by rapid elevation of glucose concentrations in blood. Goat C was rendered hypercalcemic for 4 hours by i.v. injections of calcium levulinate 60 mg/kg. at intervals of four hours and a direct TmC determination was performed. One week later the goat was given parathyroid extract (100 U.S.P. units per ml. E. Lilly & Co.) i.m.

0.01 IU a day for two days, 0.01 IU twice on the 3rd day and 0.01 IU early in the morning of the 4th day when another direct TmG determination was carried out.

In goat D infusion of a solution in water of 0.6% NaCl and 0.83% ethylene diamine tetraacetic acid (EDTA) was administered i.v. for two hours at a rate of 1 ml/min, followed by a direct TmG determination. One week later the goat was parathyroidectomized, and on the second postoperative day another direct TmC determination was carried out.

By glucose titration the tubular reabsorption of glucose is determined under increasing filtered loads of glucose. The fasting goat was anesthetized with nembutal (30 mg/kg i.v.) intubated, and put on a respirator with atmospheric

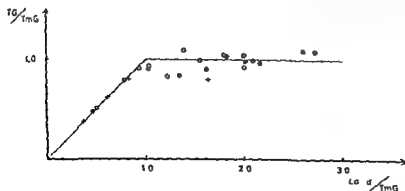


Fig. 1. Glucose titration curves in three normal goats demonstrating a maximal tubular reabsorptive capacity for glucose. TmG tubular reabsorption of glucose (mg/min). TmG maximum reabsorption of glucose (mg/min). Load filtered load of glucose (mg/min).

Table I Glucose titrations and TmG determinations in three normal goats

	BG (mg/ 100 ml)	GFR (ml/min)	TG (mg/ min)
Goat A (67-143)			
Glucose titration	68	120.6	82.0
	121	124.4	148.9
	186	119.8	211.2
	252	112.2	211.9
	335	113.8	209.6
	435	118.8	250.8
	476	112.5	232.2
	TmG = 230.9 mg/min		
Goat B (67-169)			
Glucose titration	74	128.1	92.6
	140	125.3	172.2
	184	117.5	183.2
	242	116.4	175.3
	308	114.1	187.3
	348	110.1	217.1
	420	101.8	206.6
	468	95.4	200.0
	526	88.7	200.0
	588	94.3	243.3
	632	91.1	237.4
	TmG = 211.1 mg/min		
Direct TmG determination	554	99.4	228.2
	584	92.6	215.3
	606	95.8	225.7
	614	99.3	223.4
	TmG = 223.2 mg/min		
Direct TmG determination	610	90.0	200.0
	640	80.9	206.2
	662	84.8	235.0
	698	73.1	204.5
	TmG = 211.4 mg/min		
Goat C (67-138)			
Glucose titration	84	77.4	64.8
	113	112.5	124.1
	159	89.1	127.1
	198	95.9	154.6
	233	71.3	114.3
	282	77.0	134.6
	319	87.0	144.2
	364	76.2	128.2
	402	71.5	137.0
	457	54.4	146.0
	TmG = 138.0 mg/min		

BG = blood glucose concentration GFR = inulin clearance
TmG = tubular maximum reabsorption of glucose

air. Anesthesia was maintained by nembutal (5 mg/kg i.v.) when the goat showed signs of awakening. Inulin 100 mg/kg was given i.v. followed by infusion of a solution in water of 0.6% NaCl and 0.3% inulin at a rate of 125 ml/min for 30 min. The infusion was continued with solutions of glucose in water in increasing concentrations (5.5 to 12%) and a constant concentration of inulin (0.4%) at a constant rate (0.2 ml/kg/min). Urine was

collected through a bladder catheter at intervals of 15 min and venous blood was drawn at the 5th min of each clearance period.

For the direct TmG determination i.v. infusion of a solution of 5.5% glucose and 0.3% inulin in water was given at a rate of 20 ml/min for 30 min and continued at a rate of 8.5 ml/min for an additional 90 min. From the 60th min three clearance periods of 15 to 70 min were performed.

Methods for determinations of inulin and glucose in blood and urine have been described recently (2). Serum calcium concentrations were determined by the method of Wilkinson (4).

RESULTS

The results of glucose titration studies demonstrate a well-defined tubular maximum reabsorption as measured in three goats (Fig. 1). This maximum was reached at load/TmG < 1.5. There were no significant deviations between TmG determined by glucose titration and by the direct method (Table I) nor between two control determinations in any goat (Table II). TmG was not affected by widely spontaneous changes of the glomerular filtration rate in goat C. Following injections of calcium and parathyroidectomy TmG decreased significantly but was raised by EDTA and parathyroid extract.

DISCUSSION

Sherwood et al. (3) beautifully demonstrated the parathyroid response to i.v. calcium and EDTA in goats as measured by radioimmunoassay. Administration of calcium was immediately followed by decreased amounts of measurable parathyroid hormone in blood while EDTA had the opposite effect. In this study calcium administration and parathyroidectomy led to a decreased tubular reabsorptive capacity for glucose while EDTA and parathyroid extract increased TmG. The changes of TmG were not caused directly by alterations of the blood calcium concentrations as decreased as well as increased values of TmG were observed in both hypo- and hypercalcemic states.

These observations support the previous assumption of a positive relationship between the tubular reabsorptive capacity for glucose and the parathyroid function.

ACKNOWLEDGMENT

This investigation was supported by grants from the Danish Foundation for the Advancement of Medical Science.

Table II *Direct TmG determinations in two goats before and after induced alterations of the parathyroid function*

	Serum calcium (mg/100 ml)	BG (mg/ 100 ml)	GFR (ml/min)	TmG (mg/min)
Goat C (67-138)				
Control	9	591 640 694	43.6 40.9 43.9	124.9 121.6 139.6
			42.8	128.7
Control	9.3	583 672 748	63.9 60.4 55.3	140.8 152.5 140.7
				144.4
		Mean TmG = 136.4 mg/min		
Calcium administr	10.9-10.7	739 790 858	37.8 36.5 35.0	107.4 109.3 116.9
				Mean TmG = 111.2 mg/min
Parathyroid extract	10.7-10.9	609 674 738	55.4 53.2 46.3	181.2 205.6 183.8
			51.6	Mean TmG = 190.2 mg/min
Goat D (67-148)				
Control	9.4-9.1	571 630 677	58.9 55.5 49.2	134.4 126.5 124.0
			54.9	128.3
Control	8.9	718 807 908	53.0 49.9 41.3	119.8 105.0 121.5
			48.1	115.1
		Mean TmG = 121.9 mg/min		
EDTA	6.9	584 698 740	6.1 56.8 53.3	149.1 170.6 144.4
				Mean TmG = 154.7 mg/min
Parathyroidectomy	6.8	596 626 652	42.5 37.0 35.6	101.1 95.0 101.1
			38.4	Mean TmG = 99.1 mg/min

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THE DIAGNOSTIC VALUE OF DETERMINATION OF TUBULAR REABSORPTIVE CAPACITY FOR GLUCOSE IN PARATHYROID DISEASE

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Abstract Previous observations of changes of tubular reabsorptive capacity for glucose (TmG/GFR ratio) as a consequence of parathyroid disorder are supported by the results of a follow-up study of an additional forty five patients. A distinct separation by TmG/GFR ratio exists in patients with hyper- and hypoparathyroidism. In five of thirty three patients with hyperparathyroidism (15%) the values of TmG/GFR ratio were within mean \pm s.d. of the values obtained in twenty four control patients. Parathyroid extract increases and removal of parathyroid adenoma or suppression of the parathyroid function by iv calcium decreases the ratio.

The value of a clinical application of this physiologic phenomenon is discussed. The ratio might well point to the actual parathyroid function. An increased ratio however does not illustrate whether a hyperparathyroidism is autonomous secondarily stimulated or due to secretion of a parathyroid hormone like substance. And a decreased ratio does not exclude hyperparathyroidism since three patients with hyperparathyroidism due to water clear cell affection showed low values of the ratio. Finally other factors than parathyroid hormone influence the reabsorptive capacity for glucose.

In excluding coexisting hyperparathyroidism in hypercalcaemic sarcoidosis and probably in separation of idiopathic hypercalcaemia into a renal form with stimulated parathyroid function and a renal form with suppressed parathyroid function determination of the TmG/GFR ratio might be valuable.

Recent observations indicate a positive correlation between tubular reabsorptive capacity for glucose and the parathyroid function (6, 7, 8). The purpose of this report is to elucidate this physiological phenomenon in an extended number of patients with parathyroid disorder and to discuss the value of a diagnostic test of the parathyroid function based on the effect of parathyroid hormone on the tubular reabsorptive capacity for glucose.

MATERIAL AND METHODS

The material consists of seventy patients (Table I). Thirty seven patients had hyperparathyroidism verified during

operation and subsequent histological examination of the removed glands. Eight patients had hypoparathyroidism, one idiopathic and seven postoperative, substantiated by the finding of subnormal serum calcium concentrations for several years after strumectomy. All seven patients were euthyroid. One patient had severe uremia. Twenty four patients with no signs of endocrine or metabolic disorder served as controls.

Determination of glomerular filtration rate (GFR) and maximum tubular reabsorption of glucose (TmG) were carried out in all patients. In seven of the hyperparathyroid patients the determination was repeated within 4 to 11 days after successful removal of parathyroid adenomas. In seven patients (two controls, four hypoparathyroid patients and one patient with severe uremia) the determination was repeated following intramuscular administration of parathyroid extract, 150-200 IU twice a day for four days (Para Thor Mone® 100 USP parathyroid units per ml, Eli Lilly & Co.). In one of the control patients the TmG determination was repeated after iv administration of calcium levuliste 15 mg/kg body weight during 10 hours.

In five patients, two of whom were in the postoperative state following removal of parathyroid adenomas, the TmG determination was continued for 2-3 clearance periods following iv administration of 80 units of parathyroid extract.

Observations in twenty five of the patients have been published previously (7). The results are related according to a correction of the glucose reabsorption factor (vide infra) and to the demand of a load TmG between 15 and 25 for all the hyperparathyroid and control patients.

Some minor changes of methods previously described have taken place.

Determinations of GFR and TmG were performed in the morning in all 70 patients. In the fasting state venous blood was drawn for determination of blank values of urea and fasting glucose concentration. A minor meal (1 slice of white bread, 1 cup of tea or milk) was allowed. Adequate hydration was achieved by the administration of 1000 ml of water by mouth during one hour before the investigation. Bladder catheterization was not performed. Immediately before the investigation the patients were fasted and on this urine blank values for glucose and urea were obtained. Infusions of a solution of 30% glucose and 0.3-0.6% urea in water (con-

Table 1 Serum calcium levels, urea clearances (GFR) and maximum tubular reabsorption of glucose (TmG) in patients with parathyroid disorder compared to control patients with no signs of endocrine disease

Pat	Sex	Age (y)	Se-calcium (mg/100 ml)	GFR (ml/min)	TmG (mg/min)	TmG/GFR
<i>Hyperparathyroid patients</i>						
I A	♀	59	14.6	91.0	419.0	4.61
E H	♂	49	15.0	78.0	355.0	4.25
J T	♂	45	11.3	22.8	72.9	3.20
A N	♂	54	10.9	132.5	420.7	3.19
A H J	♀	58	16.6	57.2	182.3	3.17
G M P	♀	57	14.7	30.0	93.4	3.11
P B	♀	45	11.3	129.2	389.8	2.99
I B	♀	53	15.7	29.1	86.0	2.96
K S	♀	60	10.9	99.1	284.7	2.87
M L	♂	58	10.3	100.9	289.9	2.87
M E J	♀	68	12.1	62.9	177.3	2.83
M R	♂	41	16.3	39.7	110.3	2.81
N K C	♂	62	10.2	101.7	283.7	2.77
E K J	♀	57	11.5	65.8	181.8	2.76
E E	♀	59	10.6	123.7	338.2	2.74
E A	♀	66	11.7	63.3	171.6	2.72
K N	♀	54	15.9	36.0	96.7	2.68
S P	♀	55	11.7	69.4	184.7	2.67
M E S	♀	23	11.8	46.7	124.4	2.66
M E	♀	47	13.5	110.9	288.9	2.61
C S	♀	55	11.0	137.0	359.6	2.60
K L	♀	54	10.4	121.3	310.7	2.56
R A	♀	42	11.3	82.6	208.2	2.54
G I	♀	54	14.4	74.6	189.8	2.54
H M	♀	57	10.8	102.4	258.8	2.53
E C	♀	53	15.5	60.1	150.6	2.52
J M	♀	29	11.4	149.3	339.5	2.47
V C	♀	42	10.8	63.1	155.1	2.46
E J	♀	49	9.6	73.4	177.0	2.42
V M	♀	64	11.5	81.2	179.5	2.21
G P	♀	47	11.0	147.3	322.8	2.19
A R	♀	30	10.6	70.4	149.8	2.13
B S H	♀	24	10.5	99.9	193.7	1.94
Mean		50.6		83.4		2.78
<i>Hypoparathyroid patients</i>						
L O	♀	18	8.6	112.4	192.4	1.71
M A H	♀	66	8.8	54.4	95.1	1.70
E J	♀	47	7.4	50.9	85.0	1.67
E T	♀	29	8.8	85.5	141.6	1.66
—	—	—	15.9 ^a	58.8	49.7	0.85
P E O	♀	44	7.2	48.4	72.1	1.49
E B H	♀	51	11.3	31.5	29.0	0.93
M H	♀	42	9.2	118.1	103.8	0.88
I O	♀	59	9.6	83.6	72.1	0.86
Mean		44.5		71.5		1.31

Pat.	Sex	Age	Diagnosis	Se-calcium (mg/100 ml)	GFR (ml/min)	TmG (mg/min)	TmG/GFR
<i>Control patients</i>							
K J	♀	54	Pyeloneph chr	9.5	50.5	128.0	2.52
J G	♂	61	Prost. displ. v	9.6	112.6	277.2	2.49
O S	♂	57	Spondylosis	9.2	136.0	310.7	2.29
S E	♂	40	Cystus renes	9.3	21.7	49.1	2.27
F B	♂	48	Hernia	9.5	116.5	265.8	2.26
A A	♀	44	Gastritis	9.5	113.0	247.4	2.19
V U	♂	53	Hernia	9.5	102.5	224.8	2.19
I M	♀	40	Pyeloneph chr	9.2	78.9	168.1	2.13
N N	♀	50	Psychosis		99.6	205.3	2.06

Table I (continued)

Patient	Sex	Age	Diagnosis	Se-calcium (mg/100 ml)	GFR (ml/min)	TmG (mg/min)	TmG/GFR
H G	♀	24	Normal	9.6	98.7	201.8	2.03
G O	♀	60	Pyelonephr chr	9.4	25.9	51.7	2.01
L A H	♀	27	Neurosis hyst	9.5	101.9	203.6	2.00
W M	♂	18	Normal		156.0	312.7	2.00
E I N	♀	61	Tachycardia paroxysmica	9.2	80.6	159.8	1.98
C S	♂	54	Resect ventric antea	9.9	146.3	289.0	1.98
H C	♀	58	Myositis nuchae	9.5	103.4	205.4	1.96
A E H	♀	53	von Willebrandt	9.4	111.8	215.9	1.94
K S	♀	23	Anorexia nervosa tractata	9.8	102.8	198.1	1.94
P C	♂	74	Pyelonephr chr	9.7	24.6	48.1	1.91
H O	♂	42	Observatio	9.8	96.5	183.5	1.90
A M	♀	27	Psychoneurosis	9.5	112.3	214.3	1.90
A S	♀	32	Otosclerosis	9.0	104.4	195.0	1.89
N H	♂	4	Normal	9.3	128.7	240.9	1.87
I H	♂	27	Neurosis anxiosa	9.4	143.0	266.0	1.86
Mean		43.9			98.7		2.06
<i>Patients with watery cell hyperplasia of adenoma</i>							
A O	♂	52		14.1	83.6	151.3	1.81
---	---	---		13.6	73.2	116.0	1.58
M S	♀	52		11.3	91.6	156.0	1.71
---	---	---		11.7	94.5	146.8	1.56
R P	♂	56		14.8	36.5	42.4	1.17
<i>Other patients</i>							
T L	♀	54	Hyperparathyroidism	14.0	7.0	22.9	3.26
I R	♂	28	Uremia	9.3	7.7	12.4	1.63

* Hypercalcaemia as a consequence of overtreatment with vitamin D

concentration of insulin depending on body weight) was given in an antecubital vein at a rate of 17.5 ml/min for 1-20 min (depending on body weight). The infusion was continued with a solution of 1.4-7.0 glucose (depending on sex, age, weight and kidney function) and 0.2-0.4 insulin in water (depending on kidney function) at a rate of 8.5 ml/min throughout the investigation. The quoted doses of glucose ensured a load/TmG of more than 1.5 in all cases. All infusions were made with a constant infusion pump (10 gear finger pump, D. Dich, Hvidovre, Denmark). The patients micurated again 30 and 60 min after the start of the infusions to ensure that the bladder did not contain residual urine with high concentrations of glucose and insulin from the priming period. Following an equilibration period of 60 min, three consecutive clearance periods of 0 min were performed. Blood samples for determinations of insulin and glucose were drawn from the opposite antecubital vein at 3 min before the midpoint of each clearance period.

The patients received a total amount of liquid of 2000 ml within 3 hours which ensured a mean diuresis of more than 6 ml/min in the clearance periods in most of the patients (3-11 ml/min, mean 8.8 ml/min).

In one of the hyperparathyroid patients TmG was determined during glucose titration (that is determination of tubular reabsorption of glucose under increasing filtered

loads of glucose) as well as by the direct method. In one of the hyperparathyroid patients TmG was determined by glucose titration before and after extirpation of a parathyroid adenoma.

Glucose concentrations were determined in duplicate by a modified glucose oxidase method. The modified method used in this study has not been published previously (the method was worked out by S. E. Hansen, M.D., Institute of Biochemistry, A. Juulene Mønstervej, Copenhagen, Denmark). A short outline is therefore given below.

Reagents

- Enzyme buffer: 1.4 g of glucose oxidase (Boehringer-GOD III) 12 mg of peroxidase (5 g/ml crude) 600 ml of 1/15 M phosphate buffer (pH 7.0).
- o-diamine: 0.5 g of o-diamine 100 ml acetone.
- Enzyme reagent: 150 parts of (a) 1 part of (b).
- Perchloric acid (0.3 N): 0.56 ml perchloric acid 70% (density 1.7).
- Standard solutions: standard 1 = 9.1 mg glucose standard 2 = 18.2 mg glucose standard 3 = 27.3 mg glucose standard 4 = 36.4 mg glucose.

to each of which is added 3.3 N perchloric acid to 100 ml. The four standard solutions correspond to 100, 200, 300 and 400 mg per cent glucose in serum.

Table II Normal ranges of TmG/GFR ratio quoted from the literature

	No of pat	Glucose determined in	TmG/GFR
Smith (16)	35	Arterial blood	2.72 ± 0.45 (1 s.d.)
Miller et al (12)	76	Arterial blood	2.81 ± 0.42
Goovaerts & Lambert (5)	45	Arterial blood	2.41 ± 0.35
Rieselbach et al (14)	24	Arterial blood	3.60 ± 0.36
Henningsen & Benveniste (9, 10)	18	Venous blood	2.20 ± 0.30
This study	24	Venous blood	2.06 ± 0.19

^a Nine control patients with endocrine disorders excluded

Procedure

10 ml of perchloric acid and 0.1 ml serum is centrifuged for 10 min at 3000 rpm. 0.2 ml of the supernatant is added to 5 ml reagent (c).

0.2 ml of a solution of uric acid in water 1:100 is added to 5 ml reagent (c).

0.2 ml of each standard solution is added to 5 ml reagent (c).

0.2 ml of 0.3 N perchloric acid is added to 5 ml reagent (c) (blank value).

The reaction is allowed to continue for 60 min. The specimens are then read on a Beckman spectrophotometer at 450 m μ .

An estimate of analytical error was made by 24 determinations of glucose concentrations in 18 sera with increasing amounts of glucose (from 66 to 543 mg/100 ml). Standard deviations consistently increased from 1.4 to 4.1 mg/100 ml and variation coefficients gradually decreased from 2.4 to 0.8.

The determination of inulin in blood and urine—also in duplicate—was performed according to the method of Bojesen (4). The recovery of 16.66, 23.07, 28.58 and 33.33 mg/100 ml of inulin in serum from each patient was 100.5, 99.9, 99.9 and 100.1 as determined in duplicate in 130 patients. Standard deviations were 0.86, 1.06, 1.19 and 1.75 mg/100 ml. The analytical error as measured by the mean standard deviation of 12 determinations in duplicate of each of 6 sera containing 25 to 48 mg/100 ml of inulin was 0.32 mg/100 ml (mean variation coefficient 0.9 %).

Since glucose interferes with the colorimetric determination of inulin estimation of a conversion factor was carried out for each patient. To three sera from the fasting patient were added increasing amounts of glucose and the extinction for glucose inulin was determined. In 120 determinations this conversion factor was 1.21 ± 0.27 (1 s.d.) expressing the extinction of 100 mg of glucose as per cent of the extinction of 100 mg of inulin per 100 ml. In urine the factor was found to be 0.69. The factor as determined in distilled water, deproteinized, distilled water and deproteinized urine were also found to be half the value for serum. This difference could not be explained but had to be accepted because of its constancy. The relationship between glucose and inulin in serum in this study was about 15:1 and in urine 5:1. A conversion factor for serum of 1.21 and for urine of 0.69 indicated a correction of the measured con-

centrations of inulin in serum of 18 % and in urine of only 3.5 %. Because the glucose concentrations in urine were equal in the three groups of patients to be compared (hyperparathyroid patients mean 2795 mg/100 ml, hypoparathyroid patients 2170 mg/100 ml and control patients 2340 mg/100 ml) the measured concentrations of inulin in urine were not corrected for the interference of glucose.

RESULTS

The mean values of the TmG/GFR ratio as determined in the twenty-four control patients were 2.06 ± 0.19 (1 s.d.) (Table I). These values are somewhat lower than those quoted from the literature (Table II) because in this study glucose was determined in venous blood instead of arterial blood (13).

The mean values of the TmG/GFR ratio in patients with hyperparathyroidism were 2.78 and in patients with hypoparathyroidism 1.36 (Table I).

Low values of TmG/GFR ratio were observed in three patients with clinically evident hyperparathyroidism. An exploration of the neck revealed primary water-clear cell hyperplasia in two patients and a water-clear adenoma in one patient.

The repeated determination of TmG/GFR ratio following intravenous administration of parathyroid extract for four days in four hypoparathyroid patients and in three control patients with decreased glomerular filtration rate showed increased values in all seven cases (Table III).

Continued TmG determination for 50–70 min following intravenous administration of parathyroid extract in three patients (A.O.M.K.H. and N.N.) showed increased values of the TmG/GFR ratio. The ratio of the two postoperative patients (N.K.C. and T.H.L.) however was not increased by parathyroid extract administered intravenously (Table IV).

Table III Determinations of TmG/GFR ratio in seven patients following i.m. administration of parathyroid extract (PTE) for three to four days and in one patient following calcium infusion

Pat	Diagnosis	Se-calcium (mg/100 ml)	GFR (ml/min)	TmG (mg/min)	TmG/GFR
I R	Uremia + PTE	9.2	7.7	12.4	1.63
		9.9	9.3	23.4	2.50
G O	Uremia + PTE	9.4	25.9	51.7	2.01
		10.1	27.7	66.7	2.45
K J	Uremia + PTE	9.5	50.6	128.0	2.52
		10.5	30.8	159.5	3.15
M K H	Hypoparathyroid + PTE	8.8	54.4	95.1	1.70
		10.9	83.2	223.7	2.68
I O	Hypoparathyroid + PTE	9.6	83.6	72.1	0.86
		14.5	76.3	115.5	1.54
L O	Hypoparathyroid + PTE	8.6	112.4	192.4	1.71
		12.3	106.7	218.9	2.04
M H	Hypoparathyroid + PTE	9.2	118.1	103.8	0.88
		11.3	123.3	166.4	1.36
Mean + PTE diff			64.7	93.6	1.62
			68.1	139.0	2.25
			+ 5.3	+ 48.5	+ 38.9
J G	Prolapsed discus + calcium	9.6	112.6	277.2	2.49
		11.7	107.7	234.8	2.18

Repeated determination of the TmG/GFR ratio within 4 to 11 days after successful removal of parathyroid adenomas in seven patients showed decreased values in all seven cases (Table V)

Table IV Determinations of TmG/GFR ratio in five patients within one hour following i.v. administration of 700 units of parathyroid extract (PTE)

Pat	Diagnosis	GFR (ml/min)	TmG (mg/min)	TmG/GFR
A O	Water-clear hyperplasia + PTE	73.2	116.0	1.58
		82.2	153.5	1.87
M K H	Hypoparathyroidism + PTE	33.0	56.0	1.70
		39.2	79.7	2.0
N N	Control patient + PTE	99.6	85.3	0.6
		99.6	229.2	2.30
N K C	Postoperative hyperparathyroidism + PTE	113.7	279.5	2.46
		114.9	279.3	2.43
T L	Postoperative hyperparathyroidism + PTE	5.9	12.7	2.17
		6.1	12.2	2.00

Persisting hypercalcemia after removal of a parathyroid adenoma in this patient indicated the presence of an additional adenoma which was removed at a later operation

The TmG/GFR ratio decreased in one patient after administration of calcium (Table III)

TmG as determined directly did not deviate from TmG as determined during glucose titration (M H Fig 1) The glucose titration curves of two patients in hypoparathyroid states (M H and E H Fig 1) gave no direct evidence of an extended splay as a consequence of parathyroid hormone deficiency

DISCUSSION

Five of the hyperparathyroid patients (15%) had a TmG/GFR ratio within 2 SD of that for the control patients There are several reasons to expect such an overlap primarily the dispersal of the ratio in the control patients secondly that probably other hormones exert some influence on the tubular transport of glucose (19) Certain drugs might influence the tubular reabsorption of glucose (a review is to be published) However even with a strictly hypothetical assumption that parathyroid hormone alone determines the TmG/GFR ratio a certain overlap is to be expected Berson and Yalow (3) determined parathyroid hormone in plasma by radioimmunoassay

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CELLULAR HYPERSENSITIVITY IN SJÖGREN'S SYNDROME

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Abstract An in vitro method based upon the specific action of human salivary gland extract upon the migration of leucocytes is presented. The results obtained with this method indicate that a certain state of cellular hypersensitivity directed against the salivary glands is present in two-thirds of the patients with Sjögren's syndrome. It appears that there is no correlation between the presence of circulating antibodies and the presence of cellular hypersensitivity. Furthermore it is not possible from the presented data to conclude whether the cellular hypersensitivity has a closer relationship to the pathogenesis than the presence of circulating antibodies.

In 1958 Jones demonstrated the presence of a precipitating antibody in three patients with Sjögren's syndrome. As a result of this and the presence of increased gamma globulin in sera from 50% of the patients with the syndrome he pointed out that the aetiology of Sjögren's syndrome might be analogous to that demonstrated in Hashimoto's thyroiditis, i.e. an autoimmune mechanism. The phenomenon described was non-specific (20) and so was the complement fixing antibody demonstrated by Mosbech and Østergaard Kristensen (24). Anderson et al (1) and Block et al (6) in 1964. Bertram and Højberg (4) described a phenomenon representing a specific antibody to an antigen in the cytoplasm in the epithelium of the salivary ducts. No antibodies to components in the acini were found. These findings have been confirmed by Feltkamp and van Rossum (12). An attempt to correlate the characteristic histological findings described by Godwin (14), Morgan (22), Cardell and Curling (7) and Heusler (18) with the presence of circulating antibodies directed against the salivary glands has until now failed (5).

Consequently the interest has been focused upon cellular hypersensitivity as a possible pathogenetic

factor concerning the changes of the salivary glands in Sjögren's syndrome.

This assumption is directly supported by the infiltration of lymphocytes and other mononuclear cells in the salivary glands and indirectly by the demonstration of organ specific cellular hypersensitivity in other diseases in which autoimmune mechanisms are believed to be involved, i.e. Hashimoto's thyroiditis (31-34), colitis ulcerosa (2, 25) and glomerulonephritis (3). The purpose of the present study has been to demonstrate a state of cellular hypersensitivity directed against constituents of the human salivary glands by means of an in vitro technique. This method originally described by Rich and Lewis (26) and later applied to man by Sjöborg and Bendixen (29) seems to be able to demonstrate cellular hypersensitivity in vitro.

MATERIAL AND METHODS

The material consisted of 14 patients with Sjögren's syndrome diagnosed from the presence of keratoconjunctivitis sicca, xerostomia, changes in the oral mucosa, radiographical changes in the parenchyma of the salivary glands, histologically demonstrable chronic sialoadenitis of the benign lymphoepithelial lesion type associated with systemic symptoms pertaining to the syndrome, especially rheumatoid arthritis. It was considered sufficient if two independent symptoms were present. Eleven persons without any evidence of disease in the salivary gland or rheumatoid arthritis were used as controls.

The average age of the patient group was 55, ranging from 40-74 years. The control group consisted of persons with an average age of 44, ranging from 19-69 years.

Leucocyte migration studies and determination of antibodies were performed at the same time in each individual.

Leucocyte migration studies

The technique has been described in detail in a previous paper (29). White blood cells were obtained from the

Migration index

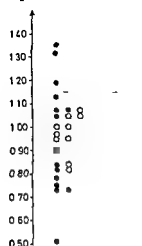


Fig 1 The distribution of migration indices in 14 patients with Sjogren's syndrome and in 11 controls. ● patient with Sjogren's syndrome ○ control

peripheral blood and after thorough washing, the cells were placed in capillary tubes then put into tissue culture chambers and tissue culture medium was added. An extract prepared from human salivary glands according to the principle described by Goudie et al (15) was used as an antigen. One hundred μ l of this extract was added to half of the cell cultures.

The cell migration areas were measured after 24 hours and the average migration area of the antigen containing cultures M was related to the average migration area of the control cultures M' and expressed as follows viz M/M' — migration index.

Thus the numerical value of the migration index expresses whether the migration has been stimulated or inhibited by the antigen. An index greater than 1.00 means stimulation and less than 1.00 inhibition of the cell migration.

Demonstration of circulating antibodies

Antibodies to the cytoplasm in the salivary duct epithelium were demonstrated by means of Coons and Kaplan's (10) immunofluorescence technique on unfixed sections (4–6 μ thick) of normal human salivary gland tissue as described by Bertram and Halberg (4).

RESULTS

Fig 1 shows the distribution of the migration indices of 14 patients with Sjogren's syndrome and 11 control persons. The observations from the patients with Sjogren's syndrome seem to fall in three categories: one characterized by inhibition of the migration, another by stimulation and a third showing neither inhibition nor stimulation. When the indices of the Sjogren's syndrome pa-

tients are regarded as a group the mean value is 0.95 and the standard deviation 0.25 while the mean value of the controls is nearly the same i.e. 0.97 but with a standard deviation of 0.09. This difference in the standard deviation means that the observations from the patients and the controls form two significantly different populations ($f=7.69$, $P<0.01$). Table I shows the findings of circulating antibodies and the corresponding migration indices of the patients with Sjogren's syndrome.

It appears that there is no correlation between the degree of cellular hypersensitivity and the presence of circulating antibody. There were no circulating antibodies in the sera from the control persons.

DISCUSSION

The alteration of the migration capacity in vitro of immunocompetent cells upon contact with specific antigen is a well established parameter of cellular hypersensitivity. The majority of experiments using different antigens and various sources of immunocompetent cells have demonstrated that the action of antigen resulted in an inhibition of the cell migration (8, 9, 11, 13, 17, 19, 23, 26, 27, 30, 33) well correlated to the delayed intra-cutaneous reaction. On the other hand a few experiments have indicated that a stimulation of the migration can also be observed (16, 21, 28, 32). The inhibition and stimulation of the migration

Table I The occurrence of circulating antibodies (cytoplasmic fluorescence) and cellular hypersensitivity (migration index) to salivary gland components in 14 patients with Sjogren's syndrome

Pat no	Cytoplasmic fluorescence	Migration index
1	+	0.52
2	—	0.70
3	++	0.73
4	—	0.75
5	+	0.78
6	+	0.82
7	+	0.83
8	++	1.05
9	+	1.06
10	—	1.06
11	—	1.13
12	+	1.19
13	+	1.32
14	+	1.35

■ to be both closely related and to be specific expressions of cellular hypersensitivity *in vitro*. This interrelationship has recently been analysed by Spborg (32) in experiments with brucella hypersensitivity. The conclusion of these experiments was that the resulting action of antigen upon the cell migration depended upon at least two factors: 1 the degree of sensitivity of the cell donor and 2 the antigen concentration. At a fixed relatively low antigen concentration a high sensitivity of the cells resulted in an inhibition, a low sensitivity in a stimulation, and an intermediary degree of sensitivity gave neither inhibition nor stimulation.

If on the other hand the sensitivity of the cells was fixed then high antigen concentrations gave inhibition and low concentrations stimulation, while intermediate concentrations apparently left the migration uninfluenced by the antigen. If the observations were regarded as a group without any subdivision according to sensitivity the highest antigen concentration always resulted in an inhibition while the distribution of indices of the lower concentrations covered the whole range from stimulation to inhibition. The non-sensitive controls were all grouped around an index of 100 with minor fluctuations at the different antigen levels.

The present results show that the migration indices of the patients with Sjogren's syndrome are spread within a wide area indicating a stimulation as well as an inhibition of leucocyte migration upon contact with antigen from the salivary glands. The distribution of the indices is similar to the distribution observed in the above mentioned brucella experiments at one of the submaximal antigen concentrations. This similarity suggests that the cells originate from persons with varying degrees of cellular hypersensitivity to an extract of salivary glands.

Because of five observations within the normal range it is impossible to say whether the total population investigated is in a state of cellular hypersensitivity. On the other hand the data presented allow the conclusion that at least two-thirds of the patients show a certain degree of cellular hypersensitivity as indicated by the *in vitro* test. Whether this percentage is the true incidence of cellular hypersensitivity in Sjogren's syndrome patients is impossible to say at the moment. The presence of stimulation as well as inhibition indicates in analogy with the brucella experiments

that the antigen concentration applied is too low. If higher antigen concentrations had been used a more precise quantitation of the cellular hypersensitivity could probably be obtained. From the data presented a certain estimate can be made of the degree of sensitivity. The patients with an inhibition of the cell migration may be considered more sensitized than the patients with indices indicating a stimulation. As regards the observations within the normal range it is impossible to predict whether these represent an intermediary degree of sensitivity or no sensitivity at all.

As previously mentioned there is no correlation between the degree of cellular hypersensitivity and the presence of circulating antibody.

The conclusion must be that humoral as well as cellular hypersensitivity is present in Sjogren's syndrome directed against components in human salivary glands.

The results show that a registration of both types of immunological reactions is necessary in order to get further information concerning the pathological mechanism involved in the changes of the salivary glands in Sjogren's syndrome.

Whether the cellular hypersensitivity is more closely related to the pathogenesis of the benign lymphoepithelial lesion in Sjogren's syndrome than is the presence of circulating antibodies has not been clarified by the experiments here presented. But it must still be kept in mind that immunological reactions and phenomena are related to many diseases without telling much about the pathogenesis of the diseases.

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THE CELLULAR CONTENT IN NON TIMED SPECIMENS OF URINE

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Abstract The present investigation is a report on the cellular content in non-timed midstream specimens of urine from 643 ambulant males and females and was performed in connection with an examination of blood pressure in the population of Bergen Norway. Special regard was paid to the influence of the specific gravity of the urine and to bacteriuria (including bacterial contamination <10 bacteria/ml).

Specimens from males and females without previous urinary tract disorders contained ≤ 78 non-disrupted erythrocytes/mm³ in approximately 95% of the cases and this value was therefore assumed to be a suitable upper limit for normal counts in routine work. The number of erythrocytes was found to be lower in specimens with specific gravities below about 1.010 than above. Hypotonic haemolysis being the probable cause of the difference.

Correspondingly ≤ 13 leucocytes + non-squamous epithelial cells/mm³ were found in approximately 95% of the sterile specimens from males and females without previous urinary tract disorders. Sterile specimens from females did not contain more cells than specimens from males but specimens with bacterial contamination contained slightly greater numbers of cells than sterile specimens. In males greater numbers of leucocytes + non-squamous epithelial cells were recorded in specimens at high than at lower specific gravities. In females high cell counts were most frequently observed at the lower specific gravities. Less than 10 leucocytes + non-squamous epithelial cells were recorded in about half of the specimens from individuals with bacteriuria. A definite borderline between normal and pathological counts can therefore not be established.

In females bacteriuria at the level 10^4 – 10^5 bacteria/ml was most often observed in specimens at specific gravities >1.019 . An inhibitory effect on bacterial growth in urine of high specific gravity is suggested.

The distinction between normal and pathologically increased numbers of cells in urine is important in clinical work. Previous investigations indicate that ≤ 5 erythrocytes/mm³ are present in urine from most healthy adults (23–27) and children (1–21) and <10 white cells/mm³ in

76–100% of specimens from healthy adults (3–7) and children (14–21, 24–25). These values have therefore been regarded as the normal upper limits. Higher normal limits for white cells have also been suggested: 12 cells/mm³ (17), 20 cells/mm³ (1), 25 and 50 cells/mm³ in newborn boys and girls respectively (16–19), 5 and 50 cells/mm³ in males and females respectively (4). Various selections of materials and various methods of collecting and examining specimens may account for the differences.

In the case of ambulant patients non-timed specimens of urine are most easily obtainable and a knowledge of the limits of normal and pathological cellular content in such specimens is therefore a point of practical importance. The object of the present investigation was to study the cellular content per mm³ in non-timed midstream specimens of urine from ambulant persons without and with previous or present urinary tract disorders. Special attention was paid to factors that might influence the cell counts such as urinary concentration (specific gravity) and the presence of bacteria (contamination or true bacteriuria).

MATERIAL AND METHODS

An epidemiological investigation of blood pressure in the population of Bergen provided an opportunity of studying the cellular content in urine specimens from ambulant persons.

Histories of previous and present symptoms and diseases were obtained by a nurse according to a questionnaire and were completed in connection with clinical examination by an internist. Special regard was paid to cardiovascular and urinary tract disorders (cystitis, pyelonephritis, urolithiasis, dysuria, pollakiuria, proteinuria including proteinuria during pregnancy). Routine examination of genitals was not included (76).

Midstream specimens of urine were collected after in-

Table I Age distribution of the material (281 males 362 females)

Age groups (y)	Males		Females	
	(No)	(%)	(No)	(%)
23-32	48	17.1	65	17.9
33-42	48	17.1	66	18.2
43-52	72	25.6	80	22.1
53-62	69	24.6	84	23.2
63-72	44	15.7	67	18.5

struction by an experienced nurse. Cleaning of genitals was performed before voiding by drawing each of three sterile swabs soaked with water and soap once over the urethral orifice: males after retraction of prepuce; non-menstruating females from front to back after having spread the labia with one hand. Females passed their urine in standing position, holding the labia apart. The procedure was not supervised unless the nurse judged it necessary or the person asked for assistance.

Protein, sugar and bacteriological examinations of the urine were carried out. Haematuria examinations not being made unless Heller's test was positive. One portion of the urine reserved for bacteriological examination was immediately placed in a refrigerator, usually being cultured the following day. Identification of bacteria was performed according to standard technique and quantitative culture by spreading 0.5 ml of a 1:100 and 1:10 000 dilution of urine on blood agar. Colony counts were made 24 hours later. If ≥ 10 bacteria/ml had been recorded a second specimen was obtained usually after 2 weeks occasionally later.

During a part of this study the numbers of non-disrupted erythrocytes, disrupted or partly haemolysed erythrocytes, leucocytes and non-squamous epithelial cells per mm urine were counted by the author. Counts were made on uncentrifuged and unstained specimens in 1 mm in an improved Neubauer haemocytometer. Specific gravity and pH were determined. Nothing except the sex was known about the persons whose urine was examined until the completion of the study.

The present material consisted of 643 consecutive specimens (from 281 males and 362 females) submitted for the first bacteriological examination, a second specimen from 42 cases which on the first examination had bacteriuria (≥ 10 bacteria/ml) and from 16 cases which on the first count had ≥ 21 red or white cells/mm. The age distribution of the individuals is shown in Table I.

In the analysis of the material a heterogeneous group of 43 males and 25 females with sterile or bacterially contaminated urine ($< 10^4$ bacteria/ml) but with various conditions that might influence the cellular content in urine was excluded because the subgroups became too small to permit conclusions to be drawn. This group comprised individuals with proteinuria, glucosuria, diabetes, cardio-vascular disorders, hypertension (systolic blood pressure ≥ 200 mm Hg, diastolic ≥ 100 mm Hg), chronic bronchitis, and those taking drugs continuously. The rest of the material was divided into three groups.

Group I

One hundred and sixty three males and 112 females who had never had symptoms or signs of disorders related to the urinary tract nor diseases that might influence the urinary tract. Their urine did not contain protein or sugar and was sterile or contained < 10 bacteria/ml (regarded as contamination).

Group II

Seventy-one males and 187 females giving a history of previous symptoms or signs related to the urinary tract but no other diseases that might be assumed to influence the urinary tract. As in group I, urinalysis revealed no pathological findings. On the basis of the available information further differentiation was not justifiable.

Group III

Four males and 38 females with bacteriuria (≥ 10 bacteria/ml) on the first examination. Other pathological conditions might, or might not be present.

RESULTS

Group I

The specific gravities of urines from males ranged from 1.001 to 1.028, from females from 1.003 to 1.029. The percentage distribution of specific gravities is shown in Table II. A bimodal distribution apparently occurred in females with a greater number of specimens at specific gravities ≤ 1.010 than in males.

The pH values of urines from males ranged from 5.0 to 8.5, in females from 4.8 to 8.4. The percentage distribution of pH values is shown in Table III.

The percentage distribution of erythrocytes

Table II Percentage distribution of specific gravities in non timed specimens of urines from 163 males and 112 females without previous or present urinary tract disorders (group I)

Spec. grav.	< 1.005	1.006-1.010	1.011-1.015	1.016-1.020	1.021-1.025	> 1.026
Males (%)	4.9	8.6	13.5	35.0	26.4	11.7
Females (%)	8.0	23.2	17.0	24.1	20.5	7.1

Table III. Percentage distribution of pH values in non timed specimens of urines from 163 males and 112 females without previous or present urinary tract disorders (group I)

pH	<5	5.1-5.5	5.6-6.0	6.1-6.5	6.6-7.0	7.1-7.5	>7.6
Males (%)	12	31	44.2	28.8	18.4	3.1	1.2
Females (%)	5.4	8.9	45.5	27.3	14.3	2.7	0.9

(non disrupted, disrupted and total) and leucocytes, non squamous epithelial cells and white cells combined is shown in Table IV.

In routine work an upper limit of cell content comprising 95% of the specimens may be regarded as a suitable normal limit. Specimens from 96.9% of the males and 94.6% of the females contained ≤ 7 non-disrupted erythrocytes/mm³. When disrupted and partly haemolysed erythrocytes were included <12 and <11 erythrocytes/mm³ were found in specimens from 95.1% of males and 95.5% of females respectively.

The upper limit of a normal white cell count should be based on the cell content in sterile specimens, since specimens with bacterial contamination (diphtheroids, micrococci $<10^4$ coli or proteus/ml) contained greater numbers of white cells than sterile specimens from females, while sterile specimens from females had approximately the same cell content as specimens from males (Table V). Sterile specimens from 94.7% of males and 95.1% of females contained

≤ 13 leucocytes + non squamous epithelial cells/mm³.

Bacterial contamination was noted in 12 specimens (7.4%) from males and in 51 specimens (45.5%) from females. About half of the specimens containing >20 white cells/mm³ were sterile.

Scatter diagrams revealed that the numbers of disrupted erythrocytes were unrelated to the specific gravity in both sexes. Smaller numbers of non-disrupted erythrocytes were noted in specimens at specific gravities ≤ 1.010 being more pronounced in specimens from males (Fig. 1) than from females (Fig. 2).

Scatter diagrams also revealed that specimens from males contained greater numbers of cells separately recorded as leucocytes and as non squamous epithelial cells at high specific gravities than at low while in females the numbers of these cells were unrelated to the specific gravities. However, leucocytes are not always easily distinguished from non squamous epithelial cells in unstained specimens. The relation between

Table IV. Percentage distribution of various kinds of cells in non timed specimens of urines from 163 males and 112 females (group I)

Cells/mm ³		0	1	2	3	4	5	6	7	8	9	10	11-20	>21
Erythrocytes non disrupted	Males	39.3	27.0	13.5	6.1	3.7	2.5	1.8	1.2	0.6	0.6	0.6	0.6	2.5
	Females	44.6	5.0	12.5	6.3	3.6	2.7	0	0.9	0.9	0	0	1.8	1.8
Erythrocytes disrupted	Males	13.5	76.4	19.0	19.0	9	2.5	4.3	3.1	0.6	0.6	0	1.8	0
	Females	16.1	4.1	17.0	1.5	6.3	10.7	7	1.8	3.6	0	2.7	1.8	0.9
Erythrocytes total	Males	7.4	16.6	16.0	17.8	11.7	11.0	3.7	1.2	2.5	1.2	3.7	4.2	3.1
	Females	8.0	20.5	17.0	11.6	8.9	4.5	8.0	3.6	2.7	7	4.5	5.4	2.7
Leucocytes	Males	37.4	31.3	13.5	3.7	5	1.8	1.8	1.8	0	0	1.2	3.7	1.2
	Females	35.7	17.9	10.7	8.9	4.5	2.7	1.8	4.5	0.9	0.9	0	4.5	7.1
Non-squamous epithelial cells	Males	48.5	24.5	12.3	8.0	3.1	1	1.2	0	0.6	0	0	0.6	0
	Females	48.2	22.3	14.3	6.3	3.6	0.9	3.6	0	0	0	0	0.9	0
Leucocytes and non squamous epithelial cells	Males	24.5	25.2	17.8	11.7	4.3	1.8	2.5	0.6	1.2	1.8	1.8	4.3	2.5
	Females	24.1	18.8	10.7	9.8	9.8	2.7	0	0.9	2.7	4.5	3.6	5.4	7.1

Table V Comparison of the percentage distribution of leucocytes+non squamous epithelial cells/mm³ in sterile and bacterially contaminated (<10⁴ bact/ml) urine from males and females without (group I) and with (group II) previous urinary tract disorders

Group No of persons	Males				Females			
	Sterile		Contam		Sterile		Contam	
	I	II	I	II	I	II	I	II
	151	62	12	9	61	115	51	72
Cells mm ³								
0-5	84.8	82.3	91.7	77.7	82.0	86.1	68.6	56.9
6-10	8.6	9.7	0	11.1	8.2	6.1	15.7	22.2
11-20	4.0	3.2	8.3	0	6.6	3.5	3.9	8.3
>21	2.7	4.8	0	11.1	3.3	4.3	11.8	12.5

these cells combined and the specific gravity of the urine is shown for males in Fig 3 and for females in Fig 4. At specific gravities <1.010 none of the specimens from males (22 in all) but about half of the specimens from females (17 out of 35) contained >3 white cells/mm³. At higher specific gravities >3 cells/mm³ were found in about a fourth (34 out of 141) of the specimens from males and about a third (25 out of 77) of the specimens from females. Of the specimens containing >3 white cells/mm³ only three specimens from males but about half of the specimens from females revealed bacterial contamination. Sterile and bacterially contaminated specimens with various cell content were intermingled at all specific gravities. In specimens

from males the higher white cell counts were consequently observed only at the higher specific gravities in specimens from females most frequently at low specific gravities.

Scatter diagrams revealed no relation between the numbers of red or white cells and the pH values of the urines.

Comparison of the numbers of non disrupted erythrocytes and leucocytes+non squamous epithelial cells in the age groups 23-47 and 48-72 years revealed only minor differences (Table VI).

Group II

Comparison of the percentage distribution of cells in this group with the distribution of cells in group I revealed good agreement (Table VII).

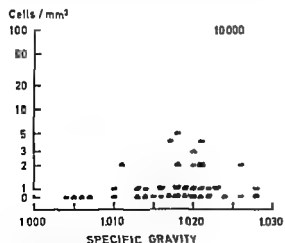


Fig 1 Relation between erythrocytes (non-disrupted) per mm³ and the specific gravity of the urine from 163 healthy males (group I).

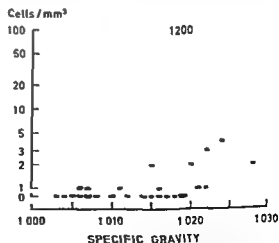


Fig 2 Relation between erythrocytes (non-disrupted) per mm³ and the specific gravity of the urine from 112 healthy females (group I).

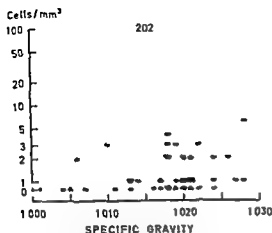


Fig 3 Relation between leucocytes + non-squamous epithelial cells/mm and the specific gravity of the urine from 163 healthy males (group I)

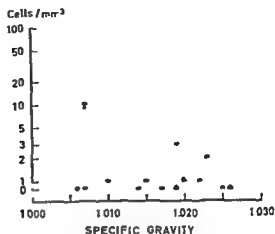


Fig 4 Relation between leucocytes + non-squamous epithelial cells/mm and the specific gravity of the urine from 112 healthy females (group II)

Bacterial contamination was noted in specimen from 12.7% of the males and 38.5% of the females. The distribution of leucocytes + non-squamous epithelial cells in sterile specimens from males and females respectively in group I and group II agreed fairly well while bacterially contaminated specimens from females contained greater numbers of white cells in group II than in group I (Table V). The number of contaminated specimens from males was too low to allow valid comparison of the groups.

Group III

On the first examination four specimens from males contained $\geq 10^4$ bacteria/ml, two of them $> 10^6$ bacteria/ml. *E. coli* was cultured in three specimens, enterococci in one specimen. The numbers of leucocytes + non-squamous epithelial

cells in these specimens were found to be 12, 26, 43 and 332 cells/mm³ respectively. Similar results were obtained on examination of the second specimen.

Thirty-eight specimens from females contained $> 10^4$ bacteria/ml (Table VIII) in twelve of these cases a negative history of urinary tract disorders had been obtained. A negative history was also obtained in five of the 19 cases containing $> 10^6$ coli/ml in both specimens. *E. coli* or species from the coliform group of bacteria were found in 35 cases, in three cases *M. m.* or *P. haemolyticus* streptococci or staphylococci were recorded. On the second examination some weeks later five specimens which on the first examination had been infected with coli and the three specimens which had been infected with cocci were either sterile or contained $< 10^4$ bacteria/

Table VI Percentage distribution of erythrocytes (non-disrupted) and leucocytes + non-squamous epithelial cells/mm in urine in the age groups 22-47 and 48-72 years (group I)

Age group No. of persons	Erythrocytes				Leucocytes + non-squamous epithel			
	Males		Females		Males		Females	
	22-47 95	48-72 88	22-47 96	48-72 56	22-47 95	48-72 88	22-47 56	48-72 56
Cells/mm								
0-5	93.7	89.7	94.6	94.6	85.3	85.3	78.6	73.2
6-10	4.1	5.9	1.8	1.8	5.3	11.8	10.7	14.5
11-20	0	1.5	1.8	1.8	6.3	1.5	1.8	8.9
> 20	1	2	1.8	1.8	3.2	1.5	8.9	5.4

Table VII *Percentage distribution of leucocytes + non squamous epithelial cells/mm³ in urine of group II and group I*

Group No. of persons	Males		Females	
	I 163	II 71	I 112	II 187
Cells/mm ³				
0-5	85.3	81.6	75.9	74.9
6-10	8.0	9.9	11.6	12.3
11-20	4.3	2.8	5.4	5.4
>21	2.5	5.6	7.1	7.5

ml. With the exception of the three specimens infected with cocci (contamination?) and one infected with coli all specimens with $\geq 10^4$ coli/ml were obtained from females older than 45 years.

One special factor concerning bacteriuria and the specific gravity of the urine was noted. In specimens with $10^4 - < 10^5$ coli or coliform bacteria/ml 13 out of 15 specimens had specific gravities ≥ 1.019 while in specimens with $\geq 10^5$ coli or coliform bacteria/ml 17 out of 50 specimens had specific gravities ≥ 1.019 (Table VIII).

Table VIII *The number of leucocytes + non squamous epithelial cells/mm³ and the corresponding specific gravity of the urine at various levels of bacteriuria (coli or cocci) in 38 females*

x indicates specimens infected with cocci

Bact./ml	First count		Second count		
	Cells/mm ³	Spec. grav.	Bact./ml	Cells/mm ³	Spec. grav.
$10^4 - < 10^5$	5	1.013	$< 10^4$	12	1.015
	5	1.022	$10^4 - < 10^5$	11	1.020
	6	1.023		36	1.021
	38	1.022		38	1.022
	4	1.011	$10^5 - < 10^6$	10	1.017
	55	1.024		15	1.006
	79	1.019		0	1.014
	80	1.023		32	1.022
	6040	1.020		81	1.023
	9	1.021	10	8	1.014
$10^5 - < 10^6$	3x	1.023	$< 10^4$	2	1.015
	4x	1.023		2	1.016
	7	1.028		30	1.030
	3	1.020	$10^5 - < 10^6$	3	1.019
		1.007		17	1.004
	4	1.017		9	1.022
	16	1.023		33	1.015
	4	1.005	10^6	34	1.011
	28	1.006		97	1.007
$> 10^6$	4x	1.004	$< 10^4$	0	1.008
	8	1.019		38	1.016
	14	1.028		27	1.027
	22	1.013		10	1.017
	8	1.021	$10^5 - < 10^6$	8	1.023
	6	1.020	$10^6 - < 10^7$	5	1.017
	51	1.016		20	1.022
	93	1.006		81	1.005
	1	1.005			
	3	1.016	III	0	1.008
	4	1.009		14	1.010
	5	1.015		3	1.003
	8	1.018		3	1.006
	15	1.014		6	1.016
	20	1.014		21	1.011
	38	1.019		1	1.017
	62	1.018		64	1.023
	63	1.023		7	1.012
	149	1.022		802	1.021
				79	1.023

The majority of the pH values in the specimens were 5.6–6.5 three specimens had $\text{pH} > 7$. There was no relation between bacteria/ml and the pH of the urine.

Ten or less leucocytes + non squamous epithelial cells/mm³ were recorded in almost half of the specimens (Table VIII). Scatter diagrams revealed no relationship between cells/mm³ and bacteria/ml or between cells/mm³ and specific gravity of urine.

DISCUSSION

In the present material ≤ 7 non disrupted erythrocytes/mm³ were recorded in about 95% of non timed midstream specimens of urine from males and females without previous urinary tract disorders (group I). The smaller numbers of non disrupted erythrocytes in specimens of specific gravity ≤ 1.010 (Figs 1–2) were most probably due to hypotonic haemolysis (11). Such specimens may be unsuitable for quantitative estimation of non-disrupted erythrocytes when these are excluded < 8 non disrupted erythrocytes/mm³ were found in about 95% of the specimens from males and females. Reismann (23) assumed that > 7 erythrocytes/mm³ might be regarded as pathologically increased.

Though 7–8 non-disrupted erythrocytes/mm³ may be a suitable upper limit for normal counts in routine work the variables of the method must be kept in mind. The random variability of haemocytometer counts implies that if e.g. 8 cells/mm³ is an estimate of the true number succeeding counts of new samples from the same specimen may vary approximately between 3 and 16 cells in 95% of the occurrences (5–10). Moreover the variable shape of erythrocytes in urine (9) results in considerable observer differences in distinguishing between cells recorded as non disrupted erythrocytes and erythrocytes with a leaky or disrupted appearance. In cases of doubt repeated examinations of the same specimen and of new specimens are necessary. In group I ≥ 21 erythrocytes (total)/mm³ were recorded in specimens from five males and three females, some of these counts being very high. In a second specimen all but one contained < 8 erythrocytes/mm³. Further clinical examination may elucidate the significance of the higher counts but this is beyond the scope of this presentation.

In agreement with previous reports (6–28) greater numbers of non disrupted than leaky or disrupted erythrocytes were found in urines from individuals without previous urinary tract disorders (Table IV) but again the observer differences in distinguishing leaky and disrupted erythrocytes from cellular debris of other origin may be large.

The observer error in distinguishing leucocytes from non squamous epithelial cells may also be large but the error in counting is probably smaller when these cells are reported together (10). In about 95% of sterile specimens from males and females without previous urinary tract disorders (group I) ≤ 13 leucocytes + non squamous epithelial cells/mm³ were recorded which therefore may be regarded as a suitable upper limit for normal counts in routine examinations. This limit was lower than for the whole series (Table IV) which also included bacterially contaminated specimens. Again the random variability of haemocytometer counts must be considered (10). If for instance 13 cells/mm³ is an estimate of the true number about 7–22 cells may be found in 95% of the occurrences on examining succeeding samples from the same specimen (5).

In females greater numbers of white cells were generally found in bacterially contaminated than in sterile specimens (Table V) but of urines with ≥ 21 white cells/mm³ specimens from all four males and two of eight females were sterile. In the second specimen three males and three females had < 10 white cells/mm³ the respective numbers of white cells/mm³ in the two counts being 80 and 68 in one male, 26 and 15, 31 and 27, 43 and 52, 64 and 54 in four females. From one female a second specimen was not obtained. It is difficult to accept such results as normal. *Trichomonas vaginalis* would probably be recorded as white cells in the cooled specimens. Other unrecognized disorders might also be present but further studies were beyond the scope of this presentation.

The urinary white cell count is of special interest in relation to the diagnosis of infections. A high number of cells is the rule in acute infections in chronic pyelonephritis normal cell content has been reported by several authors (15–18, 20–22). Table VIII shows that ≤ 10 leucocytes + non squamous epithelial cells were recorded in about half of the specimens with ≥ 10

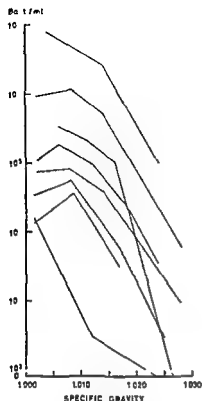


Fig 5 Rate of bacterial growth in urines of various specific gravities. Eight urines diluted with distilled water. Each dilution of the individual series was inoculated with the same amount of *E. coli* suspension; the amounts used in the different series being varied. Incubation at 37°C for 770 min.

bacteria/ml. Determination of the excretion of leucocytes is not a suitable test for bacteriuria but it may be a good screening test for urinary tract disease (8).

Although the main object of this investigation was to obtain an estimate of the cell content in non timed specimens of urine, other points relating to bacteriuria and specific gravity of urine have emerged. The lower bacterial counts (10^4 – <10 bacteria/ml) were almost always made in specimens with specific gravities ≥ 1.019 (Table VIII). A similar observation was made in a previous study (12) when the same amount of an *E. coli* suspension was added to various dilutions of sterile urine in distilled water; the concentration of bacteria in various dilution series being varied. When quantitative culture was done after incubation at 37°C for 270 min, optimal growth occurred at specific gravities of about 1.010; at the highest specific gravities the growth

was apparently retarded or inhibited (Fig 5). The pH values ranged from 5.3 to 6.6. Similar observations have been made by Asscher et al (2) while Goldberg (13) reported that forcing fluid in preparation for bladder aspiration was associated with a fall in bacterial counts due to dilution. The time factor and the amount of bacteria present beforehand may account for the difference. In the evaluation of a low bacterial count in urine, the specific gravity of the urine must be considered.

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CRYSTALLINE DIHYDROTACHYSTEROL (DYGRATYL®) IN THE TREATMENT OF HYPOPARATHYROIDISM

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Abstract Crystalline dihydrotachysterol has been used in the treatment of 26 patients with postoperative hypoparathyroidism. The clinical experience has been favourable. Dihydrotachysterol has advantages compared to calciferol, being faster in onset and fall-off of its effect, and can be given in an exact dosage. Hypercalcaemia seems to be common and urinary excretion of calcium should be determined regularly as well as serum calcium.

Hypoparathyroidism has usually been treated with calciferol (vitamin D) or AT 10. Calciferol has the therapeutic disadvantage of an effect which is slow in onset and particularly in fall-off due to the long biological half life. AT 10 consists of a mixture of irradiated sterols dihydrovitamin D₂ II—the isomer of dihydrotachysterol—being the chief component (8) and is standardized in equivalent amounts of dihydrotachysterol. This standardization may vary by a factor of five (4).

It has been demonstrated in animals that crystalline dihydrotachysterol is more effective in raising serum calcium than calciferol (1) and more important has a more rapid effect and a considerably shorter biological half life. These observations have been followed by clinical studies and crystalline dihydrotachysterol has been successfully used in the treatment of hypoparathyroidism (4, 5), vitamin D deficiency (7, 6, 3) and vitamin D resistant rickets (5). The following report is concerned with our experience of crystalline dihydrotachysterol in the treatment of postoperative hypoparathyroidism.

MATERIAL

The clinical material consists of 23 females and three males between 22 and 75 years of age (Tables I and II). Three of the patients had had total parathyroidectomy

because of generalized parathyroid hyperplasia. Six had had total thyroidectomy and 13 had had bilateral subtotal thyroidectomy. Two patients had had total hemithyroidectomy and subtotal thyroidectomy on the contralateral side with the lower part resected. In all patients the diagnosis of postoperative hypoparathyroidism was established on the basis of low serum calcium, often in combination with increased serum inorganic phosphate (Fig. 1). In 19 patients urinary excretion of calcium was determined and in three of these a roentgenologic survey of the abdomen was performed with regard to renal calcifications (Table II).

Crystalline dihydrotachysterol (Dygratyl from Philips through AB Ferrosan) was administered in tablets containing 0.1 and 0.2 mg.

Serum and urine calcium was determined with Eppendorf's flame photometer. Serum phosphate was determined as inorganic phosphate soluble in acid (2).

RESULTS

In the beginning of the study small initial dosages (0.2–0.4 mg/day) were given which were gradually increased. In the later part a high initial dosage (1.0–2.0 mg/day) was given to achieve normal serum calcium more rapidly. When a normal serum calcium had been obtained the dosage was decreased until a satisfactory maintenance dosage was arrived at. The serum calcium was normalized in all patients at a maintenance dose of crystalline dihydrotachysterol between 0.2 and 2.0 mg (Table II). With these dosages hypercalcaemic episodes of short duration were observed in two patients (Figs 2 and 3).

Hypercalcaemia which was defined as a urinary excretion exceeding 15 mEq/24 hours was observed in seven of the 19 patients in whom determinations were performed. In three of the seven patients with hypercalcaemia roentgenologic

Table I The clinical material with regard to type of surgery and clinical diagnosis

Operation	Diagnosis	No of pats
Bilateral subtotal thyroidectomy	Non toxic goitre	3
	Toxic goitre	12
Total thyroidectomy one side + subtotal contralateral	Non toxic nodular goitre	4
Total thyroidectomy	Chronic thyroiditis	3
	Non toxic nodular goitre	3
Total parathyroidectomy	General parathyroid hyperplasia	3
		46

surveys of the abdomen were made and did not reveal any renal calcifications

Nine patients had previously been treated with calciferol. All nine declared that the treatment with crystalline dihydrotachysterol was easier to

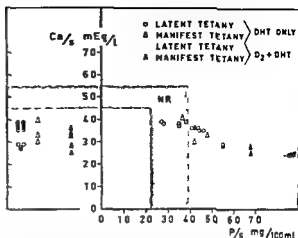


Fig 1 Serum calcium ($n=76$) and serum calcium correlated to serum phosphate ($n=19$) before treatment of postoperative hypoparathyroidism was started either with calciferol (closed symbols) or crystalline dihydrotachysterol (open symbols). Circles indicate patients with latent tetany and triangles patients with frank tetany. Normal range for serum calcium 4.5–5.5 mEq/l for inorganic phosphate 2.2–3.9 mg/100 ml

Table II Duration of treatment, maximum and maintenance dosage, urinary calcium excretion and serum calcium at time of determination of urinary calcium

Duration of treatment (months)	Pat.	Sex	Age (y)	Maximum dosage (mg)	Maintenance dosage (mg)	Ca/u (mEq/24 h)	Ca/s (mEq/l)
3	A K	-	59	12	0.2	—	—
4	L F	-	34	16	12	—	—
5	G O	-	37	0.8	0.8	11.9	4.3
5	A A	-	55	1.0	0.8	15.3 12.2	5.4 5.0
6	A M	-	28	2.0	2.0	17.6 ^a	4.7
8	K D	-	40	1.6	1.6	18.2 19.1 17.5 ^a	4.6 4.6 4.6
8	E A	-	56	0.8	0.8	2.3	5.2
9	S R	♂	44	0.4	0.4	5.0	4.9
10	T S	♂	22	1.3	0.8	14.5	4.9
11	S S	-	75	0.8	0.8	—	—
12	S Z	-	51	0.4	0.4	6.3	4.5
19	E S	-	43	1.4	1.0	17.8 19.0	5.3 4.9
20	E A	-	41	0.5	0.5	11.6	4.5
20	K M	-	30	0.8	0.8	10.1	4.8
21	A L	-	65	0.8	0.8	18.0	4.7
27	A G	-	62	0.8	0.8	18.7	4.6
27	S A	♂	50	1.2	1.2	18.6	5.4
29	E N	♂	47	1.0	1.0	14.0	4.8
29	R O	-	50	1.0	1.0	21.6 19.3 ^a	4.1 5.0
29	H P	-	30	0.6	0.6	11.0	4.5
29	W H	♂	35	0.5	0.5	6.3	4.8
29	E N	-	74	0.4	0.4	—	—
30	S B	-	39	0.6	0.5	—	—
32	S D	-	62	0.6	0.5	—	—
34	I C	-	33	0.4	0.4	—	—
34	A H	-	52	0.5	0.3	11.0	5.1

^a Patients who have had a roentgenologic survey of the abdomen, they showed no signs of renal calcification

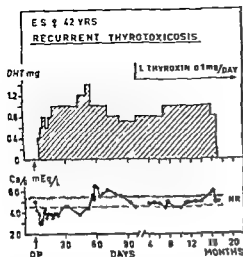


Fig 2 The course of treatment with crystalline dihydrotachysterol in a patient with postoperative hypoparathyroidism after second operation for thyrotoxicosis. Note the rapid onset of the calcium mobilizing effect and the rapid fall in serum calcium with reduced dosage. Normal range for serum calcium 4.5-5.5 mEq/l.

support and gave them a greater sense of well being. However, only four had previously been in a reasonable steady state on calciferol. The calciferol dosages used in these four patients were 0.5, 1.2, 1.4 and 1.5 mg/day. A steady state was obtained with a dosage of dihydrotachysterol of 0.4, 0.8, 0.5 and 0.6 mg/day respectively.

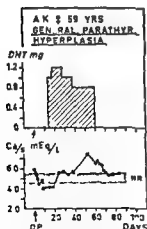


Fig 3 The initial course of treatment with crystalline dihydrotachysterol in a patient from whom four enlarged parathyroids had been removed. Note the rapid fall in serum calcium when medication was stopped. Normal range for serum calcium 4.5-5.5 mEq/l.

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Dihydrotachysterol is administered in tablets c
rather than a liquid solution. This is apt to reduce
the number of mistakes regarding dosage. The
patients generally found the tablets more con-
venient.

Crystalline dihydrotachysterol has been found
to be more toxic in animal experiments than cal-
ciferol (1). This toxic effect was established on
the basis of increase in serum creatinine and did
not seem to correlate directly with the serum cal-
cium level. A number of the patients reported
here had hypercalciuria (>15 mEq/24 h) in
spite of a normal serum calcium and without
biochemical signs of renal damage. Consequently
urinary excretion of calcium should be determined
regularly and hypercalciuria should suggest a de-
crease in dosage of dihydrotachysterol.

In this study only patients with hypoparathyroidism were included. It seems, however, that dihydrotachysterol can replace calciferol in vitamin D deficiency (6, 3) and vitamin D resistant rickets (5). Harrison et al (5) even observed that dihydrotachysterol is superior to calciferol in patients with steatorrhea. This observation may indicate that the gastrointestinal resorption of dihydrotachysterol is less affected than that of calciferol in patients with steatorrhea.

EVALUATION OF ATROMID S (CLOFIBRATE) IN HYPERLIPIDEMIC STATES

INTERIM REPORT

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Abstract Atromid S has been evaluated in an uncontrolled trial on a clinical material of hyperlipidemic states comprising 354 individuals, including an unusually high number of cases of essential hypercholesterolemic xanthomatosis. Follow-up periods varied between 1 and 4 1/2 years. Side-effects, reasons for withdrawal, persistency of lipid-lowering effects, rates of mortality and attacks of myocardial infarctions have been discussed. A significant and persistent lipid-lowering effect was seen in all varieties. Although moderate, this was also apparent in severe hypercholesterolemic xanthomatosis.

Mortality and infarction rates were low—as was also the frequency of side-effects. No serious side-effects were seen. The drug is considered to merit a considerable extension and prolongation of the study.

Both Atromid (clofibrate with androsterone) and Atromid S (clofibrate) have in a great number of reports shown a reliable and fairly potent lowering effect on the serum triglycerides as well as a moderate effect on serum cholesterol. Most of these reports include fairly small numbers of patients belonging to different clinical subgroups and have only covered a limited period. Only the large British multi-centre trial reported by Green and Margetts (10) was of a size and duration sufficient to justify an attempt to evaluate rates of reinfarction and deaths and to compare them with a number of previous materials. Their material showed a promisingly low mortality and reinfarction rate. The conclusions of the authors were cautious owing to their lack of a control material of their own.

Our experience covers a material of 354 patients followed for six months to four and a half years. Although our study has neither the size nor the duration to give a definite idea of the long-term prognosis, we have considered an in-

terim report of some interest for the following reasons:

1 The material includes an unusually large number of cases of essential hypercholesterolemic xanthomatosis. This group has been considered comparatively resistant to the action of Atromid S.

2 The inclusion of 110 clinically asymptomatic cases with essential hypercholesterolemia and essential hyperglycetemia should make an evaluation possible of the feasibility of using the drug on a primary prevention basis before clinical symptoms appear. This would also give an idea of the numbers and reasons for drop-outs in a less urgently motivated group.

3 Our own earlier long-term study on the effect of strict diet covering observation times of 5-17 years in 112 hypercholesterolemic patients on a strict diet showed a strikingly low mortality as compared with an individually matched control series (13). Due to the surprisingly low mortality in absolute terms in the strict dieters, there might even be a possibility that the addition of Atromid S to diet would change mortality in an undesirable direction. It was thought that the present series would possibly give a preliminary idea about this and the results might then influence our future policy. The reasons for running this study as an uncontrolled trial will be taken up in the discussion.

REVIEW OF POSSIBLE MECHANISMS OF ACTION OF ATROMID S ON PLASMA LIPIDS

Although not pertinent to the main purpose of the present paper, it may be appropriate to review shortly a

Table 1 Distribution of material according to age sex lipid disturbance absence or presence and character of symptoms

	Asymptomatic		Angina pectoris		Myocardial infarction		Two or more myocardial infarctions		Various clinical states		Sum
	<50	>51	<50	>51	<50	>51	<50	>51	<50	>51	
Males											
Hypercholesterolemia	3	6	3	11	6	13	—	2	1	2	47
Xanthomatosis	3	1	1	17	2	5	2	—	—	2	3
Sum	6	7	4	23	8	18	2	2	1	4	75
Hyperglyceridemia	9	7	8	13	15	16	—	4	4	5	83
Borderline lipid values	3	—	—	3	3	5	—	1	1	2	18
Total	18	14	12	39	26	39	4	7	6	11	176
Females											
Hypercholesterolemia	5	42	1	6	1	11	—	2	3	5	91
Xanthomatosis	8	4	3	10	2	4	—	1	—	4	36
Sum	13	46	4	36	3	15	—	3	3	9	127
Hyperglyceridemia	4	13	1	7	4	8	1	—	—	2	40
Borderline lipid values	1	1	—	3	—	—	—	—	4	2	11
Total	18	60	5	46	7	18	1	3	7	13	178

few facts that have emerged about the possible mechanism of action of Atromid S

Ethyl α -p-chlorophenoxy isobutyrate (CPIB Atromid S) has a plasma half life of about 1 hour. The chloride substitution in the paraposition protects the compound from hydrolysis.

Thorpe (34) found CPIB in the rat to give a definite increase in the ratio of labelled thyroxine between liver and plasma. He explained this by a displacement effect, as CPIB seems to be attached to the same binding site on albumin as thyroxine. A number of the effects of CPIB seem to be explainable by an increased metabolically active concentration of thyroxine in the liver. However this does not seem to explain all the known effects of the drug. In contrast to thyroxine which stimulates the release of FF₁ from adipose tissue CPIB has been found to give a fairly strong and long lasting inhibition of the FF₁ release from the adipose tissue. The serum FF₁ level shows a moderate but significant lowering.

CPIB is known to have a more marked lowering effect on the serum triglyceride than on the serum cholesterol.

In perfusion studies on the isolated rat liver using C palmitate Duncan et al (6) found about 50% decrease of the secretion rate of triglyceride from the liver under the action of CPIB. In hyperglyceridemic human subjects Spritz (30, 31) found a marked shortening of the plasma T₁ for C labelled triglyceride glycerol in the very low density beta lipoproteins. This was interpreted as an increase of the rate of removal of triglyceride from plasma affected by CPIB.

Hood et al (1) have earlier reported an increase of the lipoprotein lipase activity found attached to the beta lipoprotein separated chromatographically in cold. There was some inconclusive evidence that this might be due to suppression of inhibition of lipoprotein lipase.

Persson and Hood (6) found an increase in the lipoprotein lipase activity of human adipose tissue only in

three of fourteen subjects under the action of CPIB. This has been studied against the background of the rather moderate variability shown on repeated biopsy samples from 76 control subjects.

Two groups Hestel et al (22) and Hollander and Chobanian (11) using *in vivo* experiments on hypercholesterolemic human subjects have found evidence which would suggest either inhibition of the biosynthesis of cholesterol or decrease in the utilization of freshly synthesized cholesterol for the formation of lipoproteins. Isotope dilution calculations were interpreted as showing a decrease of the available pool of cholesterol in the study of Hollander and Chobanian (11).

OTHER EFFECTS OF ATROMID S

Other effects of Atromid S of possible therapeutic interest include a decrease of platelet adhesiveness (3, 8, 9), a decrease of plasma fibrinogen levels (5, 3), and an increase of fibrinolytic activity (3). The relations of these various effects to each other and to the effect on the serum lipids seem not yet to be understood in any detail.

CLINICAL MATERIAL

The material consists of 354 patients observed from six months to four and a half years.

Table 1 gives information about the distribution of the clinical material according to sex, age, type of lipid disturbance, absence or presence and character of cardiovascular symptoms. The heavy representation of cases in the decade 51-60 is seen. There were few cases below 40 and very few above 70 for which reason the material was divided into age groups <50 and >51. 4 per cent were <40, 9 per cent >51 years of age. In the group of essential hypercholesterolemia there were 11 cases of

Table II Observation periods Numbers of withdrawals deaths and infarctions in the three major clinical groups in time relation to start of Atromid S

Months	1-6	7-12	13-18	19-24	25-30	31-36	37-42	43-48	49-54	55-60	Total
<i>Asymptomatic</i>											
Taking Atromid S											
No of pat.	77	76	88	53	32	11	12	10	5	4	77
Deaths	—	—	—	—	—	—	—	—	—	—	—
Infarction	—	—	—	—	—	—	—	—	—	—	—
Stopped Atromid S											
No of pat.	16	8	3	2	3	—	1	—	—	—	33
Deaths	—	1	—	—	—	—	—	—	—	—	1
Infarction	—	—	—	—	—	—	—	—	—	—	—
<i>Angina pectoris</i>											
Taking Atromid S											
No of pat.	80	79	76	56	34	21	9	8	4	—	80
Deaths	2	—	—	—	2	—	—	—	—	—	4
Infarction	1	—	1	—	—	—	—	—	—	—	2
Stopped Atromid S											
No of pat.	6	3	5	1	1	—	—	—	—	—	18
Deaths	—	1	—	—	—	—	—	—	—	—	1
Infarction	—	—	—	—	—	—	—	—	—	—	—
<i>First myocardial infarction</i>											
Taking Atromid S											
No of pat.	62	60	54	47	29	18	13	4	2	—	67
Deaths	1	1	3	—	—	—	—	—	—	—	5
Re infarction	—	2	2	—	—	—	—	—	—	—	4
Stopped Atromid S											
No of pat.	8	7	4	3	1	—	—	—	—	—	23
Deaths	—	—	—	—	1	—	—	—	—	—	1
Re infarction	—	—	—	1	—	—	—	—	—	—	1

xanthomatosis of which 33 had widespread deposits in the tendons of all four extremities. The grading used is given below.

Xanthomatosis grade I Isolated xanthomata from the size of half a pea to a hazel nut in one or two dorsal tendons of a hand.

Xanthomatosis grade II Hazel nut sized xanthomata or bigger in the dorsal tendons of both hands and in both Achilles tendons.

Xanthomatosis grade III Huge deposits in the dorsal tendons of the hands and in both Achilles tendons (up to banana size) in addition large lumps in various locations i.e. osseous in the peroneal tendons and in the dorsal tendons of the feet.

The major emphasis has been placed on the three major sub-groups—*asymptomatic*, *angina pectoris* and *first myocardial infarction*.

The small clinical groups *two or more myocardial*

Table III Observation periods mortality and hospitalised infarctions in Atromid series comparison with the British multi-centre trial

	No. of pats	No of man months exposure	Death rate/100 man months	Myocardial infarction and re infarction rate 100 man months
<i>British multi-centre trial</i>				
Angina pectoris	137	No information	0.16	No information
Myocardial infarction	312	6809	0.39	0.47
<i>Present study</i>				
Asymptomatic	110	2,71	0	0
Angina pectoris	10	66	0.18	0.09
Myocardial infarction Total	105	093	0.43	0.47
First myocardial infarction	90	1849	0.27	0
Two or more infarctions	15	36	1	0

Table IV Deaths—Atromid S series

Sex	Age	Initial state						Time from myocardial infarction to start of Atromid S (months)	Months on/off Atromid S		Lipid response	Type of death		Comments
		Tendinous xanth grade	Asymptomatic	Angina pectoris	First myocardial infarction	Two or more myocardial infarctions	On		Off	Sudden		Infarction		
ON ATROMID S														
<i>Hypercholesterolemia</i>														
♀	62	0	-	+	-	-	-	5			Good	+	-	Severe coronary atheroscler
♂	67	0	-	-	+	-	192	9			Sign	+	-	Severe coronary atheroscler
♂	50	II	-	-	-	3	1	21			Good	+	-	—
♀	44	III	-	-	+	-	—	6			Sign	+	-	Severe coronary atheroscler
♂	30	II	-	-	-	3	1	1			?	-	+	Severe coronary atheroscler Fresh thrombus
<i>Hyperglyceridemia</i>														
♀	70	0	-	+	-	-	-	27			Sign	-	-	Massive cerebral haemorrhage
♂	54	0	-	+	-	-	-	30			Good	+	-	Violent exertion
♂	32	II	-	+	-	-	-	5			Good	+	-	Violent exertion
♂	37	0	-	-	+	-	2	10			Good	+	-	Violent exertion Old infarction Fresh thrombus
o	36	II	-	-	+	-	3	14			Good	-	+	Recent infarction Fresh thrombus
♂	74	0	-	-	-	2	1	1			Poor	+	-	—
♂	46	0	-	-	-	2	36	4			Good	+	-	Recent infarction Fresh thrombus
o	II	I	-	-	-	2	9	25			Sign	-	+	Old and recent infarction
<i>Border line lipid values</i>														
♂	49	0	-	-	+	-	18	18			Good	+	-	—
OFF ATROMID S														
<i>Hypercholesterolemia</i>														
♀	53	0	+	-	-	-	-	7	3		Poor	-	-	Bronchial carcinoma
<i>Hyperglyceridemia</i>														
♂	36	III	-	-	+	-	Unknown	2	10		Poor	+	-	Severe coronary atheroscler
♀	66	I	-	-	+	-	39	4	24		Poor	+	-	Old and recent myocardial infarctions Fresh thrombus
<i>Diabetic micro-angiopathy</i>														
♂	II	0	-	-	-	-	-	2	10		?	+	-	—
♂	49	0	-	+	-	-	-	4	15		Good	-	+	—

infarctions claudication cerebro-vascular lesions renal artery stenosis and diabetes have been included in the overall data given on mortality reasons for withdrawal of the drug and the study of the white cell counts

The dosage of Atromid S was usually 0.25 g/10 kg body weight. In a moderate number of cases a dosage as low as 0.1–0.15 g/10 kg body weight was used—in some cases with clear-cut effect on the serum lipids.

More than two thirds of the patients were under the direct supervision of the authors. The rest of the material was supervised by physicians with no direct active interest in lipid metabolism.

RESULTS

Table II gives information on observation times and withdrawals, deaths and infarctions. As seen drop-out from treatment occurred usually rather early after introduction.

Mortality and hospitalized infarctions

In Table III we have given the rates of deaths and hospitalized infarctions and compared with those

Table V Hospitalized myocardial infarctions in Atromid S series

Sex	Age	Initial state			First myocardial infarction	Months from previous infarction	Months on Atromid S	Lipid response
		Tendinous xanth grade	Asymptomatic	Angina pectoris				
<i>Hypercholesterolaemia</i>								
♀	59	0	—	—	+	14	23	Good
♂	57	1	—	—	+	$\frac{1}{2}$	15	Good
♂	61	1	—	+	—	—	13	Good
♀	84	1	+	—	—	—	6	Fair
<i>Hyperglycaemia</i>								
♂	4	0	—	—	+	$\frac{1}{2}$	7	Good
♂	51	0	—	—	+	1	12	Poor
♀	73	II	—	—	+	4 $\frac{1}{2}$	17	Good

Withdrawn one month before the second infarction due to anorexia

reported from the British multi-centre trial where such comparison has been possible.

The British multi-centre series of myocardial infarctions included 186 of patients with multiple infarctions ours 143. The average age in our series was higher the lipid disturbance by our method of selection certainly more severe but the percentage of males somewhat lower. No details on the severity of the lipid disturbance were available in the British report. Still where the figures lend themselves to a comparison they seem very much alike. Green and Margetts (10) found their figures for deaths and reinfarction rates to be approximately half of those given for control materials collected from the literature on long term anticoagulant trials. However in the absence of detailed documentation about the time elapsing from a previous myocardial infarction until the patients entered the various series it would be unwise to draw any firm conclusions. It may be stated however that our figures are closely comparable to those of the British multi-centre trial and that they appear promising enough to encourage enlarging the series and continuing with those on treatment.

Five patients died after withdrawal of Atromid S during a total exposure time of 1531 months making a death rate of 0.33/100 months. As about two thirds of the months of exposure were represented by asymptomatic and angina pectoris patients this figure would appear somewhat higher than the average of those obtained during Atromid S. The absolute figures for deaths in all subgroups concerned are naturally still definitely too small to allow any conclusions of real value.

Table IV provides details on the patients who died while on Atromid S or after withdrawal of the drug. One death was due to bronchial carcinoma. One female with extreme hyperglycaemia and moderately severe hypertension died of massive cerebral haemorrhage. The rest were either proven coronary or sudden deaths. It seems noteworthy that death was sudden in 13 of these 17 cases. In the ten autopsied cases coronary atherosclerosis was severe or extreme. In three there were signs of recent infarction. In five a fresh thrombus was found.

As is seen from the lower part of the table there was no tendency to aggregation of deaths in a close time relation to the withdrawal of Atromid S.

Details of the seven cases suffering from infarction which led to hospitalization have been given in Table V.

As in the cases who died there seemed to be no tendency to aggregation during the first months on Atromid S. The number of patients having had Atromid S for protracted periods for instance 2 years or more is still too limited to give any information on whether protracted treatment would tend to produce a lesser risk of infarction or death than in the early treatment period.

Withdrawal of Atromid S

Table VI shows that Atromid S had been withdrawn in 25% of the cases. The majority of these cases occurred in the first year of treatment as has been previously shown in Table II. In more than half of the cases the drug was withdrawn on

Table VI Reasons for withdrawal of Atromid S

	On advice of physician	Respon- sibility of patient	Total
Insufficient lipid reduction (includ 3 cases of chronic ethyism)	22	1	23
Anorexia gastric trouble	8	5	13
Too expensive	—	7	7
Chest pain worsened	1	3	4
Weight gain	1	—	1
Leucopenia	1	—	1
Re infarction	1	—	1
At admission to hospital	8	—	8
Lipids excellently con- trolled	1	—	1
Reason not clear	6	6	12
Various causes	3	14	17
	57	36	88
Total number treated	354		
Withdrawals	III (25)		

* These include impaired libido 1 psychic insuff
headache 4 dizziness 3 going on journey 2 palpitation 1
itching 1 ischias 1 active reluctance 2 heard that people
die of it 1

the advice of a physician. The upper part of the table lists motives that appear to us comparatively well grounded. The reasons seem to be the same as has been described in a number of publications. The reasons given in the lower part of the table seem mostly to reflect lack of motivation on the part both of physician and patient. Nearly

all those patients were under the supervision of physicians who were only loosely associated with the team actively interested in lipid disorders. In the group in which the drug had been withdrawn for reasons of insufficient lipid reduction there were four severely hypertriglyceridemic females who despite a rather substantial lowering of their triglyceride and cholesterol levels came within the range very commonly found in our material of myocardial infarction in early age groups. We did not measure S₂ 20-400 levels but tentatively considered the findings as a relative contraindication and withdrew the drug.

The eight patients whose Atromid S treatment was dropped on admission to hospital usually for reasons unconnected with their lipid disturbance or cardiovascular disease represent a well known annoying feature.

Short and long term lipid response

Oliver (24) has earlier produced good evidence that the lipid response was well maintained on Atromid S on a prolonged basis where no diet was prescribed. As a great part of our material was on a rather strict low fat + polyunsaturated fatty acid diet when Atromid S was started two further problems might possibly be answered. Would Atromid produce a further decrease of the serum lipids from the base line given on strict diet? During long term treatment with Atromid S

Table VII Short and long term lipid response to Atromid S in 60 subjects on different diets

	n	TG C	Before (mg %)	Atromid S 1-6 months		Atromid S 18-24 months	
				(mg %)	Reduction (%)	(mg %)	Reduction (%)
<i>Hypercholesterolemia</i>							
Ordinary diet	6	TG	135	97	-30	99	-7
		C	366	293	-20	310	-15
Strict diet	5	TG	117	83	-6	85	-4
		C	376	306	-19	315	-14
whereof							
Xanth I	8	TG	104	III	18	88	15
		C	318	277	-13	283	11
Xanth II	6	TG	102	91	11	93	9
		C	398	366	8	366	-8
Xanth III	5	TG	13	95	78	83	33
		C	494	339	-19	401	-19
<i>Hypertriglyceridemia</i>							
Ordinary diet	11	TG	246	153	-38	172	20
		C	331	281	15	287	-13
Strict diet	III	TG	59	152	41	144	-40
		C	321	90	-10	299	7

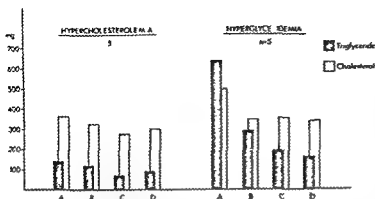


Fig 1 Serum lipid changes on strict diet as compared with a combination of strict diet and Atromid S after short and prolonged treatment A or dietary diet B low fat + polyunsaturated diet C low fat + polyunsaturated diet + Atromid S 1-6 months D low fat + polyunsaturated diet + Atromid S 18-54 months

would the lipid response remain the same as initially as a tendency to slackening of the diet might occur—although the patients were encouraged to maintain their diet?

Sixty patients who had been on Atromid S for 25-54 months (average 37 months) and whose lipid data were adequate for assessment were used for this purpose. The data have been given in Table VII and Fig 1. The reduction of lipid values in the late period (18-54 months) as compared with the early period (1-6 months) seems well maintained although a slight tendency to less reduction may be seen in most subgroups. There seemed to be no greater tendency to a rebound in those on strict diet as compared with those who were treated with Atromid S and maintaining a normal diet.

When the group of essential hypercholesterolemia cases was subdivided according to the presence or absence of tendinous xanthomata and according to the size and distribution of these lesions, there emerged no definite trend showing any marked resistance to treatment in those with severe xanthomatosis.

Thus Atromid S did produce a further lipid reducing effect from the base line of a strict diet. This effect was essentially well maintained on a long term basis in the present group of subjects. Surveying the individual data in this group of 60 subjects it seemed apparent that individuals obtaining a poor to moderate effect initially continued to do so also in the late period.

Side-effects

As has been seen previously from Table VI, we have only encountered side effects of limited importance. There were a number of cases in which

the drug was withdrawn for different usually vague symptoms not described in the great number of publications on Atromid S that have appeared. This has been interpreted as predominantly reflecting a lack of interest in the physicians responsible for instructing and informing their patients about the purpose of the treatment.

In a few patients there was an impression that chest pain worsened during treatment.

As there have been a number of thorough studies previously dealing with the effect on liver function tests, serum transaminases and coagulation parameters, we early dropped systematic following of these measurements.

Weight changes

There have been several reports of weight gain in materials of patients on ordinary diet. We had sufficient data on 68 patients followed for more than two years who were on a strict diet.

There was an average weight gain of about 2 kg. This seemed moderate as compared with earlier reports and as possible mechanisms for weight gain have been rather thoroughly discussed earlier by Macmillan et al. (18) we have refrained from further comments.

White cell counts

Prout and Edwards (27) have described a case of agranulocytosis during Atromid S treatment in which however other drugs might also have been involved. Engstrom (8) described moderate leucopenia in four out of eight subjects during a study of short duration.

One of our patients exhibited a white cell count of 2000. Atromid S was withdrawn and has not been reintroduced. A systematic study

was made in 74 subjects of the material. In a total of 412 observations on these 74 subjects the distribution was normal with a median value of 5500 w.c. Only 14 observations fell in any time below 3000 w.c. In two of these cases the low values prompted a temporary withdrawal of the drug which was later reintroduced with perfectly normal counts for prolonged periods.

In the 38 cases who have been followed systematically for more than 2 years up to 4 1/2 years there has been no sign of progressive lowering of the white cells in a single case. Mean values for the group remained very stable between 5400–6200 w.c. per mm³ throughout the whole course. There was not a single case of symptom producing granulocytopenia in the entire material.

Other factors of possible importance for evaluation of the results

Hypertension under active antihypertensive treatment was rare in this material and we have therefore refrained from analysis. None in the asymptomatic and angina pectoris groups and rather few in the group of first myocardial infarction survivors were under anti-coagulant treatment when Atromid S was introduced. In some of these the anti-coagulants were withdrawn after a period. There was no case of severe bleeding recorded in the material.

DISCUSSION

An uncontrolled study such as the present one can evidently only give answers to a limited number of questions. One may say that the drug was fairly effective in lowering serum lipids in most of the hyperlipidemic cases and that there were a number of cases with no recognizable effect. If there was a reduction of lipid values this was protracted for up to 4 1/2 years. It would also seem to be clear that Atromid S provides a further lipid lowering effect to that induced by diet. Protracted treatment did not cause any signs of late toxicity or late troublesome side-effects. When the drug was withdrawn it was done in approximately half of the cases for reasons which have been discussed in a number of earlier publications. However in approximately half of the cases in which the drug was stopped the reason would seem to have no connection with the drug and sometimes not even with the underlying

disease but seemed to be an effect of a lack of determination in the supervising physician. Well instructed asymptomatic individuals with a biochemical derangement but without the urgent motivation of severe pain or fear of death have continued with the drug for many years.

The important problem whether the mortality rate or rate of hospitalized infarctions was higher or lower than in previously existing materials can be only partly answered. Definite conclusions can evidently not be reached without a randomized control material for several years to come. Our group have had some reasons to refrain from the use of a randomized control material and a double blind placebo technique. These reasons may be stated as follows. Work on the effect of diet was started in comparatively young patients often with angina pectoris and a very strong family history of early myocardial infarction and death. Here the question of randomization was from the outset an impossible one. As observation periods increased in a number of these patients without new infarctions and angina pectoris in a number of patients disappeared or seemingly was ameliorated the start of randomization became even more questionable. We naturally recognize the impossibility of registering improvements of angina pectoris in an objective way to the satisfaction of others.

We think that the position we have taken has been strengthened by the reports on long term trials of lipid reduction. Eight of ten published long term studies on the effect of diet have reported positive results (1, 4, 13, 14, 15, 17, 20, 21). Several of these studies had obvious weaknesses including our own. However the recently published studies of Leren were extremely penetrating and thorough on strictly randomized material. Morrison's and Hood et al.'s studies had certain drawbacks but represented the longest observation periods hitherto. The two studies giving equivocal results were randomized but together with that of Koranyi (14) represented the shortest observation times of all studies, two and three years respectively. Rose's (29) two-year study was on a small material which was further subdivided into three experimental groups. It is probable that the diet method employed with addition of supplements of oil to the fat low cholesterol low diet led to the fact that only 16, 12 and 15 subjects out of the 26, 26 and 28 respectively in the initial

groups were stated to be left in the study at the end of the second year. Although the method of selection methods of assessment and the statistical work up were of a high standard to us this study remains wholly inconclusive.

The London Hospital Research Committee's study (16) with strictly randomized material on a low fat diet did not use polyunsaturated fat. The diet group lost on an average ten the control group eight pounds of weight. There was a reduction of serum cholesterol in both groups averaging about 40 mg% in the diet and 20 mg% in the control group. The curves showing the cumulative relapse rates showed for the diet group a flattening from the second to the fifth year (diminishing numbers of subjects) with only three relapses while the curve for the controls showed a steady rise. Altogether it seems clear that some definite change of habits must also have occurred in the control group. It would have been interesting to see a prolongation of this study for a further 4, 5 or 6 years in order to reach definite conclusions. Two of the three long term oestrogen studies have reported positive results (19, 33) but the well executed five year study of Oliver and Boyd (25) was entirely negative. One of the two prolonged heparin studies we have been able to find (7) also showed a reduction in death rates and reinfarction. The other one (2) showed a suggestive difference between the treated and the placebo treated group but this was not statistically significant.

A survey of the present literature then has strengthened our opinion that the use of randomized material and a double blind placebo technique in a lethal disease often associated with severe pain and not rarely with severe anxiety may be questionable. We fully realize that other approaches must necessarily give rise to considerable doubts as to the effect for several years to come. We think however that something may be gained by using other methods of assessing results. This might for instance include survival rates of consecutive cohorts of first myocardial infarction survivors studied closely before and after major changes in therapeutic approach.

Further reports may in the meantime be expected from the British multi-centre trial. The grant study on a randomized double blind placebo basis now well under way in Edinburgh may be hoped to give better information on the potential

effect of lipid reduction with Atromid S in the asymptomatic males having serum lipids in the top section of the normal population. There hardly seems to be any reason to try to copy studies of this type with limited resources. We feel it well motivated to continue treating all definitely high risk patients of the type included in the present study.

CONCLUSIONS

1 Atromid S has been evaluated in a material of 354 patients with essential hypercholesterolemia, essential hyperglyceridemia and a small group with borderline lipid values. Observation times varied between half a year and four and a half years.

2 There were no deaths or infarctions in the clinically asymptomatic group of 110 patients.

3 In the angina pectoris and myocardial infarction groups of 102 and 105 patients respectively, the rates of deaths and myocardial infarctions were closely comparable to those obtained in the British multi-centre trial.

4 In those who died while on Atromid S death was sudden in the majority of cases. In the ten autopsies a fresh coronary thrombus was found in five.

5 Atromid S produced a definite further lipid lowering effect from the base line obtained on a strict diet (low fat + polyunsaturated fat). The effect seemed well maintained over a period of up to four and a half years. Although the group of severe essential hypercholesterolemic xanthomas cases appeared somewhat more resistant there was a definite further decrease in serum lipid as compared with that obtained on a strict diet also in this group.

6 A little less than 25% stopped taking Atromid S the great majority during the first year of treatment. The most common reasons given for stopping the drug appeared to be anorexia, gastrointestinal troubles, insufficient lipid reduction and economic reasons. However in about half of the cases the drug was stopped for reasons that appeared less well founded and seemed mostly to reflect a lack of motivation in both physician and patient.

7 The drug was withdrawn in a single case because of leucopenia (2000 w.c./mm³). A systematic study of 74 patients of whom 38 were

followed for more than two up to four and a half years revealed no further cases and no progressive lowering of white cells in the group

Although no conclusions as yet may be drawn about the value of the drug in preventing deaths and infarctions the comparatively low rates recorded the safety and the fairly good lipid reducing effect appear to motivate extended use of the drug in the type of high risk individuals included in the present study It might also be considered to extend the use in groups carrying somewhat less risks

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AUTONOMIC BLOCKING DRUGS ON CIRCULATORY ADAPTATIONS AT REST AND DURING EXERCISE IN MAN

Pronethalol and Poldine in Long term Treatment

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Abstract Oral doses of Pronethalol and Poldine were given to six subjects as blocking drugs for not less than one week. A hemodynamic study was then repeated 1-3 hours after the last dose. Heart rate, intra-arterial pressures, right atrial pressure, cardiac output, pulmonary ventilation, oxygen consumption and respiratory exchange ratio were measured with the subject at rest in a recumbent and a sitting position, and during exercise sitting on a bicycle ergometer. The mean values at rest were unchanged during blockade but the reactions of the variables when the patient was sitting up were changed for systolic and diastolic pressure, oxygen consumption and arteriovenous oxygen difference. The responses of heart rate and arteriovenous oxygen difference when the subjects changed from the recumbent to the seated position had lower variabilities during treatment. The left ventricular work and stroke work in the seated position had also lower variabilities during treatment than before. During exercise the mean values for heart rate, arterial pressures, pulmonary ventilation and oxygen consumption were lower during blockade. The variabilities of cardiac output, stroke volume, left ventricular work and stroke work and arteriovenous oxygen difference were also lower. The changes in mean values from rest to exercise were reduced during treatment for heart rate, arterial pressures, left ventricular work, pulmonary ventilation and oxygen consumption, and the variability of reaction during treatment was higher for right atrial pressure but lower for left ventricular work. Similar studies which were made in each case with Pronethalol and Poldine respectively indicated that the present blockade was essentially additive. Thus the most obvious effects were produced by Pronethalol but many of the orthostatic reactions were influenced by Poldine. The exercise adaptation was well maintained during the present autonomic blockade.

The autonomic nervous system plays a major part in the adaptation of the circulation to different activities. Autonomic blocking drugs are used in the treatment of many disorders, i.e. hypertension, peptic ulcer disease.

Pronethalol, a potent β -adrenergic receptor blocking agent, has been tried in different rhythm disturbances (3, 14, 15), angina pectoris (2, 14), as well as hypertension (10, 13). Therapeutic use has been hampered by some adverse effects in laboratory animals (1, 9). This drug was used, however, as the sympathetic blocking drug in the present study, and Poldine, a quaternary ammonium compound for peptic ulcer disease, was chosen as the parasympathetic blocking drug.

MATERIAL

Five male subjects aged from 21 to 34 years were selected for the study (Table I). Four of them attended the hospital for nonbleeding peptic ulcer disease, one for slight melena, probably due to hemorrhagic gastritis, as no wound was seen at the X-ray investigation, and one for evaluation of obesity (B.B.). One subject (K.O.) had moderate hypertension, the others, judging by their history, physical examination or ECG, showed no signs of circulatory disease.

METHOD

The basal study was made in the morning in a post-absorptive state. A radiopaque catheter was introduced percutaneously into a cubital vein and advanced to the right atrium or a central vein under fluoroscopic guidance. A polyethylene catheter was also inserted in the brachial artery using the same technique (Seldinger). About 10-20 mg of lidocaine without epinephrine were used as the local anesthetic.

After a rest period of half an hour in recumbency, blood pressures were recorded, using variable inductance transducers (EMI 490 A, Flema-Schönanander AB) and a multichannel oscillograph. ECG was recorded simultaneously. Expired air collection was started via a low resistance system for about 5 min. After about 1 min of air collection, pressures were again recorded, followed by

Table I Presentation of some characteristics of the subjects

Subject occupation	Diagnosis	Age	Height (cm)	Weight (kg)	Daily dose (g) over number of days	
					Poldine	
B lift attendant	Normal subject (obesity)	26	182	130.8	0.040/8 + 0.10	
H K excavator attendant	Peptic ulcer disease	35	186	86.0	0.016/5 + 0.024/5 + 0.032/3 + 0.040/15 + 0.010	
K O cashier	Peptic ulcer disease	32	171	71.6	0.016/3 + 0.074/1 + 0.032/1 + 0.010/2 + 0.010	
G A cabdriver	Hypertension	54	181	70.0	0.016/1 + 0.024/2 + 0.037/18 + 0.008	
I O plumber	Peptic ulcer disease	33	186	100.5	0.016/4 + 0.024/2 + 0.032/2 + 0.040/5 + 0.010	
B K workshop laborer	Peptic ulcer disease	21	186	74.8	0.032/2 + 0.040/2 + 0.032/1 + 0.040/1 + 0.010	
	Hypertacidity					

a dye dilution determination of cardiac output. Brom sulphalein was rapidly injected in the right atrium and fractional arterial sampling was simultaneously started as well as an ECG.

After a rest period of half an hour these recordings were repeated with the subjects sitting in an armchair.

After a similar rest period they started an exercise while sitting on an electrically braked bicycle ergometer. Blood pressures and ECG were recorded every second minute. After 5 min of work expired air collection was started and after 9-11 min of work the cardiac output procedure was applied.

The work load of three subjects was 300 kg m/min, that of one 400 kg m/min and that of two others 600 kg m/min.

The hemodynamic study was repeated after treatment with Pronethalol and Poldine. The mean daily dosage of Pronethalol was 0.75 g given for 10 days and the corresponding dose for Poldine was 32 mg for 14 days. The last doses were given together with a small amount of water about half an hour before the catheter was inserted. These doses were 0.3 g in four and 0.1 g in two of Pronethalol and 10 in five and 8 mg in one of Poldine.

Five cm below the sternal notch was chosen as the zero pressure level in recumbency and the third intercostal space as that in the sitting position. Mean pressures were obtained by electrical integration.

The expired air was analyzed for its oxygen and carbon dioxide contents using the Scholander apparatus and oxygen consumption, respiratory exchange ratio and pulmonary ventilation were calculated.

The arterial samples were centrifuged and the dye concentrations read from a Beckman DU densitometer after alkalization. A dye concentration curve was plotted on semilogarithmic paper and the cardiac output calculated. The hematocrit was determined after centrifugation at 1600 g for 15 min. The stroke volume was calculated using the simultaneous ECG recording. The total peripheral

resistance was determined as the pressure difference between mean brachial arterial pressure and mean right atrial pressure in mm Hg over the cardiac output in l/min and expressed in arbitrary units. The left ventricular work and stroke work were calculated from the brachial arterial mean pressure times the cardiac output and the stroke volume respectively and expressed in both kg m/min and g m (grammeter). The pressures obtained immediately before the cardiac output procedure were used for all the calculations.

Mean values and standard errors were determined according to standard formulas. Individual differences for corresponding recordings were tested by *t* test. *P* values are given for these differences. Standard deviations were tested by *F* test.

RESULTS

Mean values and standard deviations as well as *P* values for differences are given in Table II.

Heart rate (HR beats/min)

No change in mean values was obtained during rest but the orthostatic reaction during blocking had a lower variability. During exercise the heart rate was lower during treatment ($P < 0.05$). The increase from rest in the sitting position to exercise was smaller during treatment, a difference which was even more marked ($P < 0.01$). The individual responses are shown in Fig. 1.

The brachial arterial pressures (P_{BR} systolic, P_{BD} diastolic and P_{BA} mean pressure in mm Hg).

The mean values at rest were not different during treatment but when the changes from a rest

and last dose	Interval after control study (days)	Symptoms during treatment
Pronethalol		
0.6/8+0.2	45	Dryness of the mouth Palpitations
0.9/10+0.3	30	Dryness of the mouth Less peptic symptoms and less apprehended during treatment
0.6/2+0.9/8+0.3	12	Dryness of the mouth
0.6/15+0.2	23	Dryness of the mouth Difficulties in starting micturition
0.3/1+0.6/2+0.9/3+0.3	12	Dryness of the mouth
0.3/1+0.6/1+0.9/2+0.6/2+0.9/1+0.3	7	Dryness of the mouth Dizziness

cumbent to a sitting position were considered a small increase in diastolic pressure occurred in the sitting position during treatment as compared with a small decrease before treatment ($P < 0.02$). An indicative change ($0.10 > P > 0.05$) in the

same direction for the systolic pressure i.e. a smaller positional change occurred but the change in the mean pressure and the pulse pressure was not significant.

During exercise all pressures during treatment were lower the decrease was most pronounced in the systolic pressure and then in the diastolic and pulse pressures. When the changes from rest to exercise were considered increases in pressure were smaller during treatment these differences were even more pronounced ($P < 0.02$ for the smallest difference). The individual pressure reactions are given in Fig. 2.

The mean values of the right atrial pressures (RA mm Hg) were unchanged but the variability of atrial pressure change due to exercise was higher while under treatment.

Flow-dependent variables

Cardiac output (Q l/min) stroke volume (SV ml) peripheral resistance (SVR mm Hg l/min) left ventricular work (LVW kg m/min) and stroke work (LVSW) (g m) at rest were unchanged during treatment.

Table II Hemodynamic data on six subjects during rest in the recumbent and the sitting position and during exercise on bicycle ergometer before (B) and during (D) treatment with Poldine and Pronethalol. Mean values, standard deviations and P value ranges for differences. For symbols see the text.

	HR		P_{BA}		P_{BAD}		P_{BA}		$P_{BA} \sim P_{BAD}$		RA		Q		SV	
	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D
Rest	71.8	72.8	142.7	137.2	78.7	79.5	99.7	101.7	64.0	57.6	3.0	2.0	8.00	7.83	118	112
Recumbent	21.4	14.6	27.3	16.4	8.2	5.7	17.4	12.2	19.4	11.4	1.2	1.7	1.7	1.4	39	35
Sitting	72.3	77.8	130.7	131.3	75.2	80.7	93.2	99.7	54.8	50.2	2.5	3.6	6.34	6.39	91	87
	13.7	12.6	19.6	11.0	8.6	4.7	10.9	9.3	12.9	6.4	2.3	1.62	1.19	3.2	22	
Exercise	157.2	107.2	172.1	144.8	83.8	75.7	109	100.0	88.8	69.2	1.7	0.7	13.4	11.0	109	104
	23.7	15.1	18.1	12.7	3.5	7.6	7.0	8.0	18.7	12.2	4.1	3.6	4.6	1.7	46	18
	< 0.05		< 0.005		< 0.02		< 0.10		< 0.01							
	SVR		LVW		LVSW		VE		VO ₂		M		(a-v) _{O₂}		Hct	
	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D
Rest	12.7	13.3	11.0	10.7	136	147	9.49	11.05	305	298	0.797	0.89	38.7	37.8	44.7	41.3
Recumbent	3.5	4.1	3.5	1.7	40	44	0.02	2.78	68	74	0.061	0.238	7.6	4.6	3.0	3.4
	< 0.025															
Sitting	15.9	16.4	8.0	8.8	115	116	11.95	11.22	313	292	0.840	0.835	54.3	45.0	45.3	4.7
	4.4	4.6	2.5	1.0	40	4	3.03	2.30	73	43	0.035	0.16	13.5	7.1	3.2	3.7
	< 0.05															
Exercise	8.8	9.3	20.1	14.9	163	142	42.62	37.54	1486	1318	0.903	0.905	119.5	105	48.0	45.0
	8	1.9	7.0	1.8	68	21	5.48	5.66	143	197	0.04	0.046	32.0	9.9	3.8	3.3
	< 0.10															

HEART RATE

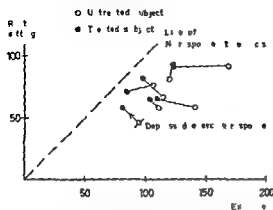


Fig 1 Heart rate reactions at rest and during exercise before and during blocking. All treatment values are nearer the identity line where no reaction to exercise is obtained.

The only effect during exercise on the mean values of these variables was an indicatively smaller increase in the left ventricular work from rest to exercise during treatment as well as a lower degree or variability also evident in the left ventricular stroke work.

During treatment there was also less variation in the exercise recordings of cardiac output, stroke volume, left ventricular work and stroke work. While the subjects were sitting at rest the variations in left ventricular work and stroke work were also smaller during treatment.

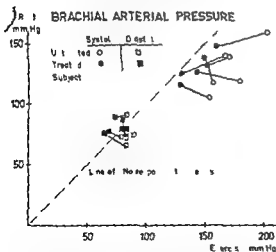


Fig 2 Arterial pressure reactions at rest and during exercise. All the treatment values of systolic pressure are nearer the identity line where no reaction to exercise is obtained. Some of the diastolic pressure reactions cross the line for treatment.

Respiratory dependent variables

The mean values for pulmonary ventilation (V_E l/min BTPS), oxygen consumption ($\dot{V}O_2$ ml/min STPD), respiratory exchange ratio (R) and arteriovenous oxygen difference ($a-\bar{v}O_2$ ml/l) were unchanged during rest. With regard to changing from a recumbent to a sitting position the small increase in oxygen consumption when the subjects were sitting up before treatment changed to a small decrease during treatment ($P < 0.10$). The arteriovenous oxygen consumption was correspondingly changed, i.e. the increase was less when the subjects sat up during treatment ($P < 0.05$). On treatment during exercise variability in the arteriovenous oxygen difference was less than before.

During exercise pulmonary ventilation and oxygen consumption were indicatively smaller during treatment. The differences in the changes from rest to exercise reached the same level.

Hematocrit (Hct) was somewhat smaller during treatment.

The subjective working capacity was the same. Dryness of the mouth was felt by all the subjects during treatment. One (B.B.) complained of palpitations and another (G.A.) of micturition difficulties. No ECG abnormalities were observed.

DISCUSSION

The present method of studying the prolonged effect of circulatory active drugs seemed to be suitable judging by the good reproducibility obtained when the hemodynamic study was repeated (11).

Pronethalol tested alone by the same method produced bradycardia and reduction in brachial systolic and diastolic pressure during exercise comparable to that obtained in the present study (13). In the present study however the reduction in the brachial arterial mean pressure was only indicative during exercise which applied also to the decrease in oxygen consumption. The same change in positional response regarding brachial diastolic pressure when the subject was sitting up was evident when Pronethalol was given alone. Variations in these responses differed being higher for heart rate and lower for systemic vascular resistance. The variabilities in left ventricular work and stroke work were also lower however after Pronethalol alone. No changes in positional re-

sponses in respect of systolic pressure oxygen consumption arteriovenous oxygen difference were observed when Pronethalol was given alone.

When considering the changes from rest to exercise Pronethalol given alone also produced a smaller increase in heart rate systolic and diastolic pressure oxygen consumption and left ventricular work ($P < 0.10$) but had no significant effect on the mean pressure.

According to another report Poldine given alone produced increased heart rate and brachial arterial diastolic pressure ($P < 0.10$) in the sitting position (12). These changes were not observed in the present study. In recumbency at rest Poldine decreased the oxygen consumption and arteriovenous oxygen difference. These changes were not detectable in the present study. There was also an indicative rise in cardiac output. Poldine alone also exaggerated the positional changes in heart rate brachial arterial mean pressure blood flow and right atrial pressure. These changes were also abolished in the present study when Pronethalol was added. Variability during Poldine treatment was less at rest in recumbency only for left ventricular work whereas in a sitting position it was also less for stroke volume. During exercise there was also less variation in these variables and in systolic and mean pressure cardiac output and stroke work.

The Poldine and Pronethalol doses given in this study were comparable with the doses administered when each drug was tested alone (12, 13).

Thus when the subject was sitting up changes in the adaptation of the circulation during this blockade of the autonomic nervous system could be explained by the β adrenergic blockade of Pronethalol. Some of these effects were however counteracted by Poldine. Most of the changes in the adaptation to work could be explained in the same way but the decrease in variability might have been due to Poldine.

When both drugs were administered arterial pressure reaction during exercise was at least as small as when Pronethalol or Poldine was given alone. This may indicate that compensatory reflex mechanisms from the other autonomous divisions are operative when one part is blocked. However the receptor part responsible for the resistance vessel reactions was not shown to be blocked by Pronethalol which makes the decrease in arterial pressure reaction difficult to explain. The active

dilatation of resistance vessels mediated by the β adrenergic system must also be blocked. This makes the pressure reaction still more difficult to explain. Some intrinsic β sympathetic activity of the drug may account for this.

A more complete blocking by drugs of the autonomic nervous system in man was investigated by Kahler et al. (8). They studied the adaptation to work in scumbeny during high peroral doses of Guanethidine and a high intravenous dose of Atropine. They obtained a smaller increase in heart rate an inversion of the arterial pressure reaction and a smaller cardiac output increase during exercise. After administration of the blocking drugs their values during exercise for stroke volume and ventricular stroke work were lower than before Atropine when given alone did not qualitatively change the response to exercise consequently they concluded that the sympathetic division plays a major role in this adaptation. However Atropine when given intravenously has initially the effect of increasing cardiac output heart rate and blood pressure (16) but later (15 min or more) the only effect observed is increased heart rate (4). This may depend on different delays after injection before blocking all the various circulatory reflex stations. Unlike Atropine Poldine is not regarded as penetrating the blood brain barrier. Consequently with Poldine only peripheral blocking of the parasympathetic influence can be accomplished.

After high doses of Atropine and Pronethalol the cardiac reactions to vagal and sympathetic stimuli were almost entirely eliminated and an increase of heart rate which was most marked in young and healthy subjects was observed during rest (6, 7). The arterial pressure rose but cardiac output was not affected (7). The term intrinsic heart rate was used to denote this autonomic "isolation" of the heart.

No similar effects on heart rate or blood pressure were seen in this long term study. The blocking doses were also not comparable.

The smaller variability of the flow reactions during exercise observed in the present material was probably an effect of autonomic blockade. This may indicate that the autonomic nervous system is not of major importance for adaptation to work. It may only reinforce or weaken the response in individual subjects and thus cause greater variability in the responses during exercise.

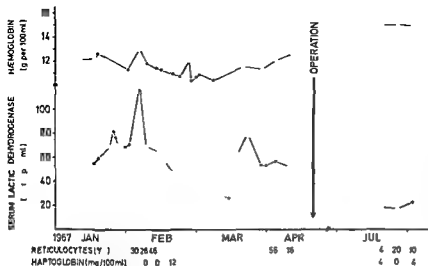


Fig 1 Hematological changes in a 48 year old woman with aortic valve disease. The hemolysis subsided after operation

At combined mediastinal and left ventricular puncture the pressure in the aorta was 174/62 mm Hg and in the left ventricle 250/42 mm Hg without any gradient across the mitral valve.

Thoracic aortography confirmed the diagnosis of severe valvular aortic stenosis with moderate aortic incompetence and heavily calcified aortic valve.

Course during admission

On admission she had a hemoglobin of 12.2 g/100 ml (Fig 1) a sedimentation rate of 27 mm/hour a white cell count of 11 200/ μ l. She had a fever rising to 39.5 C in the evening subsiding spontaneously within four days. A few coli bacteria were cultured from the urine.

One day after the right heart catheterization—which took place on the 8th day after admission—the serum lactic dehydrogenase which was then determined for the first time was 55 U/ml and continued to be elevated ranging between 110 U/ml and 47 U/ml throughout hospitalization which lasted three months (Fig 1).

The elevation was due to isoenzymes no 1 and 2 but as it was constant and neither the patient's clinical condition nor the ECG changed suspicion of myocardial damage as cause of the elevation was soon abandoned.

Four weeks after admission she again had fever up to 39.4 C lasting for 16 days and then subsiding spontaneously. No other focal signs of infection were observed except for moderate pyuria. The hemoglobin fell slightly to 11.4 g/100 ml. There were no signs of bleeding. The laboratory tests were as follows.

The red-cell count was 3 500 000/ μ l the mean corpuscular volume was 81 fl the mean corpuscular hemoglobin concentration 35 g/100 ml the hematocrit 37% the reticulocyte count 30-26-46% (Fig 1) the serum iron 46 μ g/100 ml the serum iron binding capacity 269 μ g/100 ml the serum B 480 μ g/100 ml the folic acid normal the serum haptoglobin values were 0-0-12 mg/100 ml (Fig 1) the bleeding time the coagulation time the prothrombin content and the thrombocyte count were all within normal limits.

The serum albumin was slightly decreased (3.28 g/100

ml) and the alkaline phosphatase elevated (66-61-45 U/100 ml the upper normal limit being 33 U/100 ml) the serum bilirubin was normal. The serum electrolytes and serum creatinine were within normal limits. The protein bound iodine was 6.0 μ g/100 ml and the serum cholesterol ranged between 163 and 279 mg/100 ml. The erythrocyte glucose-6-phosphate dehydrogenase was 156 pico-units per erythrocytes (normal value 88-151 pico-units).

Histologic examination of a sternal marrow specimen revealed hyperplastic normoblastic erythropoiesis.

The conclusion was that the patient had hemolysis of unknown cause. She received no drugs that could cause hemolysis. The erythrocyte osmotic fragility was normal. The direct antiglobulin (Coombs) test Ham's and Crosby's tests and direct Donath-Landsteiner test were all negative and tests for cold and heat agglutinins were negative.

Subacute bacterial endocarditis was strongly suspected as the cause of hemolysis in this patient. However negative results were obtained from nine bloodcultures (seven of which were from arterial blood) and from culture of the bone marrow and this condition was then ruled out.

It was then supposed that chronic hemolysis might be explained by mechanical damage of the erythrocytes when passing the stenotic aortic valve. On the peripheral blood smear a number of bizarre erythrocytes and fragments of erythrocytes were seen.

Operation

The cardiac condition of the patient remained unchanged in spite of diuretic treatment in addition to the treatment with digitalis and she was referred for operation.

At operation during extracorporeal circulation the ascending aorta was widely dilated measuring up to 7 cm in diameter. The outer diameter of the aortic root was 34 mm. The aortic valve was heavily calcified and there was agglutination between the left and the right coronary cusp so that the valve appeared as a bicupid valve with a split opening of 18 x 3 mm. A forcible jet was felt in the aortic arch before perfusion was started. After resection of the aortic valve the cusps were found to be about

Table I One hundred and two patients with rheumatic and congenital heart disease investigated for hemolytic anemia

	No of pats	Hemolysis
Congenital ^a	3	0
Mitral valvular disease	28	11
Aortic stenosis	13	3
Aortic insufficiency	4	0
Aortic stenosis and aortic insufficiency	10	2
Aortic and mitral valvular disease	15	2
Total	102	7

^a Except for aortic valvular disease

1 cm in thickness with uneven surface. A homograft was inserted.

The patient's clinical condition improved considerably.

Follow up

Three months after the operation renewed hematological studies were undertaken the following values being obtained at an interval of one week (Fig 1): hemoglobin 15.0-15.1-15.0 g/100 ml reticulocytes 4-7-10% lactate dehydrogenase 18-17-22 U/ml haptoglobin 4-0-4 mg/100 ml serum iron 93-91-104 µg/100 ml transferrin 241-76 = 60 µg/100 ml.

MATERIAL AND METHODS

One hundred and two patients with rheumatic and congenital heart disease admitted to this department during a four month period were investigated. The distribution of diagnosis is seen in Table I.

Seventeen of the 78 patients with isolated mitral valve disease had previously had mitral valvulotomy. The 3 patients with congenital heart disease consisted of 1 with atrial septal defect, eight with ventricular septal defect, five with pulmonary stenosis, four with Fallot's tetralogy and three with coarctation of the aorta.

The hemoglobin level, reticulocyte count, serum lactate

dehydrogenase, serum haptoglobin and plasma hemoglobin were determined at least twice with one day in between. The normal values in this laboratory are: hemoglobin males 132-176 g/100 ml, females 117-157 g/100 ml; reticulocyte count <12%; serum haptoglobin 35-170 mg/100 ml; serum lactate dehydrogenase 7-73 U/ml; plasma hemoglobin not established.

A diagnosis of hemolysis was established when a stable or decreasing hemoglobin level was found together with reticulocytosis in the absence of bleeding.

The patients who thus showed signs of hemolysis were followed for at least two weeks with frequent determinations of the above mentioned values. In patients with a mean reticulocyte count of >20% the ⁵¹Cr red cell survival half time ($Cr T_{1/2}$) was determined (normal value 72-31 days). Furthermore in the hemolytic patients a number of additional laboratory analyses were made: serum bilirubin, serum iron and serum iron binding capacity, serum B₁₂, serum folic acid, direct antiglobulin (Coombs) test, direct Donath-Landsteiner's test, Ham's and Crosby's tests, serum hemolysis at 37°C, osmotic fragility of the erythrocytes, leucocyte count and thrombocyte count. The peripheral blood was investigated for schistocytes and the bone marrow was examined.

RESULTS

Hemolysis was found in seven patients (Tables I and II). Five of the patients had isolated aortic valve disease while the remaining two had aortic valve disease in combination with slight mitral valve disease.

In none of the patients with isolated mitral valve disease or congenital heart disease apart from aortic valve disease could hemolysis be demonstrated.

None of the seven patients with hemolysis had iron deficiency, folic acid or vitamin B₁₂ deficiency. The leucocyte counts and distribution and the thrombocyte counts were within normal limits. The direct antiglobulin (Coombs) test, Donath-Landsteiner's test, Ham's test and Crosby's test

Table II Studies of seven patients with mechanical hemolysis

Pat	Diagnosis	Sex	Age (y)	Hemo- globin (mean) (g/100 ml)	Reti- culocytes (mean) (%)	Serum haptoglobin (mg/100 ml)	Serum LDH (units/ml)	⁵¹ Cr T _{1/2}
H I	AS AI	+	58	10.4	11	4-8	21	0
J O	AI MI	+	34	10.9	6	1-71-90 104-8	20	19
A A H	AS	+	52	13.9	4	0-24-90	31	19
H J	AS AI	+	56	11.6	0	0-0-4.4	19	∞
N H	AS	+	45	1.6	17	0-0-0.8 28	0	
P S	AS	+	53	15.3	17	0-4-16-12 12.8	4	
A H	AS AI MS	+	57	1.1	17	0-0-11-15	17	

AI = mitral insufficiency
AS = aortic stenosis

MS = mitral stenosis

LDH = lactate dehydrogenase

AI = aortic insufficiency

were all negative and no serum hemolysins were found. The osmotic fragility of the erythrocytes was normal in all cases.

The peripheral blood of all the seven patients with hemolysis contained a small number of schistocytes and erythrocyte fragments and the bone marrow showed hyperplastic normoblastic erythropoiesis.

Table II shows that four of the seven patients had anemia while the hemolytic disease was compensated for in the remaining three. The $^{51}\text{Cr Tl}$ was slightly decreased in three patients and was in the lower part of the normal range in one (H J). The serum bilirubin was elevated in one patient (A A H) on admission (1.8 mg/100 ml) but subsequently was normal (0.4 mg/100 ml).

Patient J O was admitted with a reticulocyte count of 42% and a serum haptoglobin value of 12 mg/100 ml. During her stay in the department where most of the time she was bedridden the reticulocyte count dropped to 12% while the serum haptoglobin value rose to 104 mg/100 ml. The hemoglobin level was stable at 10.9 g/100 ml. At a later admission from her home where she had been active in housekeeping the reticulocyte count had again increased to 48% while the serum haptoglobin value was 8 mg/100 ml.

The plasma hemoglobin level in all 102 patients was between 0 and 8 mg/100 ml; there was no difference between the values for patients with and without hemolysis.

DISCUSSION

The possibility of traumatic hemolysis in patients with unoperated valvular heart disease was first suggested by Dameshek in 1964 (5). He discussed a case of hemolytic anemia in a 63 year old woman with calcified aortic and mitral valve disease.

Thereafter the question of traumatic hemolysis in these patients was investigated with determination of ^{51}Cr erythrocyte survival time by Brodeur et al (2, 3), Yacoub et al (16) and Gehrmann et al (7).

Brodeur et al (2) found shortened $^{51}\text{Cr Tl}$ in 18 of 21 investigated patients with unoperated aortic valve disease. He states that although none of the patients was anemic the findings for one

patient were compatible with compensated hemolytic anemia. This patient however, had previously undergone aortic commissurotomy.

Yacoub et al (16) investigated 11 patients with aortic valve disease and found normal values of the $^{51}\text{Cr Tl}$.

Gehrmann et al (7) observed shortened $^{51}\text{Cr Tl}$ in 18 of 37 patients with different valvular heart diseases. Ten of the patients had isolated aortic valve disease, two had combined aortic and mitral valve disease and the remaining six had isolated mitral valve disease. None of the 18 patients was anemic and the hemoglobin level in the six with isolated mitral valve disease ranged between 14.4 and 17.0 g/100 ml.

Brodeur et al (3) observed only a slightly shortened $^{51}\text{Cr Tl}$ in four of 14 patients with unoperated isolated mitral valve disease; none of these patients was anemic.

Determination of serum haptoglobin has been used by Veneziale and associates (14) and Cullhed (4) to discover low grade hemolysis.

Veneziale and associates (14) found decreased value of serum haptoglobin in six of 24 patients with unoperated valvular heart disease. The hemoglobin value was only mentioned in respect of three of the six patients; it was normal. The reticulocyte count was not determined. Preoperatively Cullhed (4) found low serum haptoglobin values in two of 26 unoperated patients with aortic valve disease. Neither of the patients had anemia; the reticulocyte count is not stated.

In a study of serum lactic dehydrogenase level in patients before and after cardiac catheterization Jorgensen et al (9) found elevated values in ten of 43 patients with different unoperated congenital and rheumatic heart diseases. They suggest that the elevated values could be attributed to a low grade compensated chronic hemolysis.

In several other reports traumatic hemolysis has been suggested (11, 15, 17).

Miller et al (11) found traumatic hemolysis in a 27 year old man with rheumatic aortic and mitral valve disease. The degree of hemolysis as determined with $^{51}\text{Cr Tl}$ increased with increasing muscular activity of the patient.

Westring (15) reports the condition in a young woman with aortic insufficiency. In a further prospective investigation of 12 patients with aortic valve disease traumatic hemolysis was present in one anemic patient; in two other patients the ^{51}Cr

TI was slightly shortened but there was no other evidence of hemolysis.

So far four cases of hemolytic anemia have been described in patients with unoperated valvular heart disease (5 11 15). All four patients had aortic valve disease but two had in addition mitral valve disease.

The patient, with compensated hemolysis reported by Bodeur et al (2) had previously been operated on for aortic stenosis with commissurotomy. In a case described by Ziperovich and Palev (17) hemolytic anemia developed after unsuccessful mitral valvuloplasty at a second operation the anterior leaf of the mitral valve was found to be destroyed and the anemia disappeared after a Starr-Edwards valve had been inserted. This anemia corresponds to the severe hemolytic anemia developing in patients with postoperative prosthetic valve defects (6 10).

In our material hemolytic anemia was found in five patients with valvular heart disease. Furthermore three patients had compensated hemolysis. All eight patients had aortic valve disease as the predominating abnormality while two had also slight mitral valve disease.

Our findings and the previously reported cases indicate that unoperated mitral valve disease is hardly likely to provoke traumatic hemolysis.

One of our patients (10) demonstrated increasing hemolysis with increasing physical activity which occurred also in the case described by Miller et al (11).

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LACTIC ACID ACCUMULATION IN CONNECTION WITH FRUCTOSE INFUSION

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Abstract Infusion of fructose in healthy experimental subjects and in patients with diabetes mellitus is found to cause an increase in the blood concentration of lactate that is in relation to the rate of infusion. At an infusion rate of 1 g/kg BW/h or above the lactate concentration rises by 5 mEq/l or more. In healthy subjects infusion is generally associated with a relatively unappreciable rise in arterial glucose concentration. In diabetic patients on the other hand, the rise is pronounced. Clinically significant acidosis is found to be exacerbated by rapid fructose infusion. It is therefore emphasized that this form of treatment is contraindicated in acidotic states. Administration of fructose has no beneficial effect on the metabolism in insulin-requiring diabetic patients whose insulin has been temporarily discontinued. On the contrary it exacerbates their acidosis and produces a considerable rise in blood sugar.

During the past few years fructose has started to be used to a great extent instead of glucose for parenteral nutrition with carbohydrates. In recent survey articles fructose is stated to have many advantages over glucose for this purpose (e.g. 19).

Fructose is more rapidly metabolized resulting in a lower total sugar concentration in the blood than when the corresponding amount of glucose is given (2, 17, 20). It has been reported that post-operative patients and uraemic patients with glucose intolerance can utilize fructose in a normal way (6, 13). Since fructose is not dependent on insulin for intracellular penetration it has been considered of value in cases of diabetic acidosis or after total pancreatectomy (5, 16, 17). Moreover fructose is stated to have a less irritant effect on peripheral veins than glucose which implies that fructose solutions for intravenous use can be given in higher concentration (19).

An account is given in this paper of a study of the way in which infused fructose affects the lactic acid concentration in blood both in healthy

experimental subjects and in patients with diabetes mellitus. We had two aims. One was to ascertain whether significant lactic acidosis could be induced by fructose infusion. The other was to determine whether fructose had any beneficial effect on the metabolism in diabetic patients whose insulin had been temporarily discontinued.

MATERIAL AND METHODS

Seven healthy experimental subjects and three patients with juvenile diabetes mellitus were investigated in the morning after an overnight fast. An indwelling catheter was inserted in the brachial artery for blood sampling. The healthy subjects were given a continuous infusion of 10% fructose. The rate of infusion was 0.4 g/kg BW/h in one case (M A) and 1.0-1.3 g/kg BW/h in four (M V, A A, A F and E J). The infusion proceeded for two hours or longer. Two subjects (M II and M R) were given a more rapid infusion (2.2 and 3.5 g/kg BW/h respectively) but in these cases administration of fructose was discontinued after an hour in view of subjective complaints in the form of nausea and precordial pain.

The metabolism of the splanchnic region was studied by means of hepatic vein catheterization in four subjects (M H, M R, A F and E F). The results of these studies have been reported elsewhere (2).

Two of the diabetic patients had been without insulin for 77 h and the third patient (M C) for 48 h before the investigation.

M C and A L were given a fructose infusion of 1.2 g/kg BW/h during 3 h. After an interval of 90 min A L was given 0.1 U of rapidly acting insulin intravenously followed by a fructose infusion at the same rate for 3 h.

A fructose infusion (0.6 g/kg BW/h) was started in case C K. After 30 min hyperventilation appeared and she became somnolent. Rapidly acting insulin (25 IU) was given intravenously and after a dose of 20 g of fructose in the course of 5 min the infusion was stopped and bicarbonate solution administered (100 ml of 1.3% solution in the course of 50 min). A fresh attempt at infusing fructose (1 g/kg BW/h) also had to be dis-

Table I Changes in blood glucose fructose and lactate in connection with fructose infusion

Values obtained after 2 h of continuous infusion in all cases except M H and M R in which the infusion was stopped after 1 h

Subject	Sex	Age (y)	Weight (kg)	Infusion rate of fructose (g/kg BW/min)	Δ Arterial glucose concentration (mg/100 ml)	Δ Arterial fructose concentration (mg/100 ml)	Δ Lactic acid concentration (mEq/l)
<i>Normals</i>							
M A	♂	22	84	0.5		44	0.9
M V	♂	18	53	1.1	14	93	5.0
A A	♂	38	72	1.0	~13	107	5.0
A F	♀	24	59	1.0	33	64	5.5
E J	♀	22	60	1.3	61	104	6.7
M H	♂	24	70	2.2	38	178	6.5
M R	♀	22	51	3.5	35	383	8.0
<i>Diabetics</i>							
A L	♂	17	57	1.2	105	112	5.4
M C	♀	16	50	1.2	265	132	5.3
G K	♀	13	41	0.9	— ^a	75	3.7

^a Insulin administered

continued because of increasing symptoms of acidosis. During the subsequent part of the experiment insulin was administered repeatedly i.v. Fructose infusion was then restarted (0.6 g/kg BW/h) and continued for 3½ h. The blood pH and standard bicarbonate were determined at close intervals. On the day after the experiment the patient's blood sugar was 340 mg/100 ml and her alkali reserve was normal.

Blood samples for determination of fructose, glucose and lactate were taken at short intervals in every experiment. In all experiments in diabetic patients and in some of the normal cases the blood pH and standard bicarbonate were determined by the Astrup method (18).

Glucose was determined by the α -toluidine method (11). Fructose was determined with meta-aminophenol after removal of the glucose with glucose oxidase. The details of the method have been published recently (17). The blood lactate was determined enzymatically with lactic acid dehydrogenase using Boehringer's reagent kit.

RESULTS

Healthy subjects (Table I Figs 4 and 5)

In every case the initial blood lactate concentration was below 1 mmol/l. During fructose infusion the fructose concentration in blood rose accompanied by a rise in the concentration of lactate. In cases in which the pH and standard bicarbonate were also determined a fall in bicarbonate was recorded which corresponded approximately to the rise in lactate (Fig 1).

A rise in blood glucose was noted during fructose infusion in five of the seven cases. The greatest rise amounted to 61 mg/100 ml (case E J).

Diabetic patients (Table I)

Before administration of insulin all three patients had a rise in the blood concentration of fructose and lactate in connection with fructose infusion. The rise was of the same order of magnitude as when a corresponding amount of fructose was infused in normal subjects (Table I Figs 4 and 5).

In all three cases fructose infusion was also associated with a considerable rise in blood sugar.

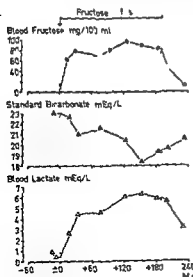


Fig 1 Fructose infusion (1.1 g/kg BW/h) to a normal subject (M V).

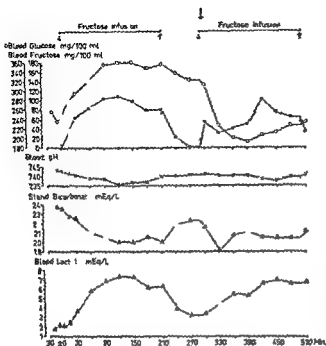


Fig. 2. Fructose infusion without and with insulin to the diabetic patient A L: 20 IU insulin intravenously.

Case A L (Fig. 2)—who was given a second fructose infusion after i.v. administration of 20 IU of rapidly acting insulin—had on this occasion as well a rise in blood fructose and lactate which after two hours was only inappreciably smaller than that in connection with the first infusion (without insulin). The rise in both fructose and lactate concentration however seemed to take place more slowly and successively during the second fructose infusion (after administration of insulin).

Case G h (Fig. 3) had at the start of the experiment pronounced metabolic acidosis with a standard bicarbonate value of 9.4 mEq/l and blood pH of 7.13. The first fructose infusion had to be discontinued since she showed clinical signs of increasing acidosis and her blood pH fell to 7.05. Insulin and bicarbonate solution had to be administered. A steady state infusion of fructose could not be given until she had received repeated intravenous doses of insulin. During the later part of the experiment the patient's blood pH and standard bicarbonate rose successively as a sign that the keto-acidosis had decreased and the blood sugar had fallen appreciably. The blood lactate concentration during fructose infusion however rose to 8 mEq/l.

When the infusion was started case M C had a standard bicarbonate value of 18 mEq/l. After

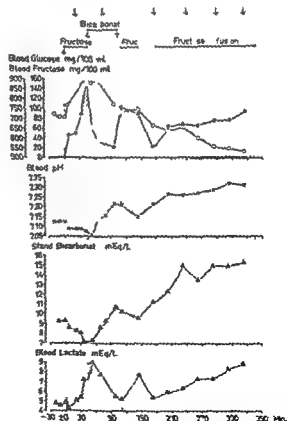


Fig. 3. Fructose infusion to the diabetic patient G h. As each 10 IU insulin was given i.v. For further details see text.

whose insulin had been discontinued for 48–72 h that the formation of lactate was significantly lower than when a corresponding amount of fructose was given to healthy experimental persons. They however used much smaller doses and shorter infusions than in our experiments.

During the initial fructose infusion without insulin all three diabetic patients had a considerably greater rise in blood sugar than that in the healthy subjects. It has recently been demonstrated by means of hepatic vein catheterization in healthy subjects that the glucose production in the splanchnic region falls during fructose infusion (2). Since this occurs despite the liver taking up more fructose than the lactic acid formed, it indicates that a considerable part of the fructose taken up in the liver is normally stored as glycogen. The much greater increase in blood sugar in the diabetic patients indicates that the glucose production in the splanchnic region increases, i.e. the part of the fructose that does not form lactic acid is metabolized to glucose in the liver instead of being stored as glycogen.

The rise in lactate which occurred during fructose infusion of 1 g/kg BW/h or more was of sufficient magnitude (5–8 mEq/l) to have a significant effect on the acid base equilibrium so that metabolic acidosis arose. The effect was particularly drastic in the diabetic patients M.C. and G.K. Even before starting the experiment they had keto acidosis. In both cases there was a pronounced fall in arterial pH in case G.K. to as low as 7.04. This patient became greatly affected clinically during fructose infusion and had to be treated acutely with repeated insulin injections and administration of bicarbonate. If fructose infusion had been continued without taking these measures it would presumably have been detrimental to the patient.

Thus our results show that rapid administration of fructose can lead to considerable lactic acid acidosis and—at any rate in patients who already have acidosis—may exacerbate their condition. We therefore conclude that rapid infusion of fructose is contraindicated in acidotic patients. The great risk should be stressed especially when the patients may be suspected to have an increased production of lactate on grounds of anoxia or for other reasons (10). Andersson et al. (1) recently observed two cases of severe acidosis in children which became further exacerbated during fructose ad-

ministration in one of them with a fatal outcome.

In view of the data presented here it is probable that formation of lactic acid during administration of large amounts of fructose did in fact contribute decisively to exacerbating the acidosis and causing the death of one of the children.

ACKNOWLEDGEMENTS

This work was supported by grants from the Swedish Medical Research Council (project no. K67 19X 1002 02 and B67 61P 2108-01) and the City of Stockholm.

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IDIOPATHIC SCOLIOSIS IN OLD AGE

I Respiratory Function¹

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Abstract Lung function was studied in a group of 14 female patients between the ages of 37 and 63 who had been diagnosed as idiopathic scoliotics about 50 years ago. The examinations comprised spirometry and determination of ventilation and the alveolar gas exchange at rest and during work. Roentgenological measurements were used to determine the degree of scoliosis; the cases being divided into two groups according to the degree of deformity. The moderately deformed group contained seven patients with an angle of less than 60° while the other seven patients made up the severely deformed group with an angle of more than 60°. The latter group had significantly lower total and vital capacities, maximum ventilation and forced expiratory volume in one second and also a significantly higher respiratory rate and tidal volume. The two cases with the most severe degree of scoliosis had an impaired alveolar gas exchange; the one with the largest angle (102°) presenting signs of respiratory insufficiency with retention of carbon dioxide during oxygen inhalation.

The results thus suggest that the degree of deformity in scoliosis is correlated with lung function.

Although vital prognosis is said to be impaired by severe scoliosis, no detailed analysis of this relationship has appeared in the literature. Nilsson and Lundgren (18) have reported, however, that the mortality in a group of patients with severe idiopathic scoliosis and an observation period of up to 50 years was twice as high as in corresponding groups of the normal population. When the study was made the mean age of the living patients was 62.1 years while for the dead patients it was 46.6 years. Heart or lung disease was the cause of death in 60% of the latter group whereas almost half of the living patients

had physical complaints which rendered them incapable of working.

The high mortality observed in idiopathic scoliosis and the predominance of heart and lung diseases as the cause of death raise the question of the cardiac and pulmonary function in scoliotic patients and the extent to which the degree of scoliosis is correlated with any impairment of these functions. The aim of the present study was to deal with the questions that can be answered by means of data from investigations of lung function.

MATERIAL

The study was concerned with 15 female patients with idiopathic scoliosis who first presented at the Orthopedic Clinic at Karolinska Institutet during the period 1913-1918. Mean age at examination of pulmonary function was 63 years. All the patients were among the subjects studied by Nilsson and Lundgren (18) with respect to long-term prognosis. Thus larger group, however, included patients living in distant parts of Sweden. For practical reasons the present study was limited by inviting only those patients to participate who lived in the Greater Stockholm area. A further element of selection was introduced by some of the patients declining to take part in the study which depended upon voluntary participation. One of the fifteen patients (no. 5) who accepted the invitation took part only in the Bernsteim spirometry. All the others completed the examinations as planned.

METHODS

Static lung volume (l BTPS) Total lung capacity and its subdivisions were determined by the helium dilution method, using a closed spirometer system. **Dynamic lung volume (l BTPS)** was determined with a modified Bernsteim Spirometer. Normal values were taken as the data

¹ A preliminary report was presented at the annual meeting of the Swedish Medical Society in 1964.

Table I Some anthropometric data on 15 patients with scoliosis

Case no	Age (y)	Height (cm)	Weight (kg)	BMR ()	W_{130} sitting (kpm/min)	Degree of scoliosis ()
1	69	165	65	-6	400	42
2	62	164	57	+3	200	67
3	63	150	60	+10	250	97
4	65	155	62	-17	300	10
5	58	150	42	—	300	85
6	57	155	47	-11	300	72
7	63	156	57	± 0	450	4
8	63	161	67	+5	400	54
9	63	147	52	-9	300	86
10	64	163	65	+14	300	15
11	57	135	42	+1	200	102
12	63	150	62	+13	200	80
13	65	159	64	-8	300	13
14	67	162	67	+5	350	48
15	63	153	63	+8	250	84
\bar{x}	63	155.4	59.5	+0.6	300	49.9
s.d.	± 3.4	± 8.43	± 8.26	± 9.51	± 75.6	± 30.6
Range	57-69	135-167	42-67	-17-+14	200-450	10-102

n = number of subjects \bar{x} = mean s.d. = standard deviation

corrected to BTPS presented by Berglund et al (3) Birath et al (5) and Grimby and Soderholm (11). Distribution of inspired gas was studied by means of N_2 elimination during oxygen breathing using a Lilly N_2 meter. The result was expressed as the time (min) for lowering $F_{R_{N_2}}$ to 0.02. Gas distribution was also measured as the helium equilibration time during spirometry. Venous admixture due to true shunts in the lungs was estimated as outlined by Berggren (2).

Expiratory gas was collected into Douglas bags and the volumes measured with a pirometer. Gas samples were analysed for oxygen and carbon dioxide by the Haldane technique and for carbon monoxide with a hopcalite CO meter (Stålevä) as described by Linderholm and Sjöstrand (15).

Arterial blood was sampled anaerobically at rest and at the end of the work load period from an indwelling teflon catheter in the brachial artery. Partial pressures of O and CO in arterial blood P_{aO_2} and P_{aCO_2} mm Hg were measured with a Clark electrode and a Severinghaus P_{CO_2} electrode respectively (21). pH and standard bicarbonate were determined by the Astrup microtechnique (1). Carotid mono-oxide in blood was determined by the method described by Linderholm et al (16). Oxygen saturation of blood S_{O_2} and hemoglobin concentration g/100 ml were measured spectrophotometrically. Dead space including a correction for breathing valve dead space of 40 ml and 50 ml at rest and during work respectively was calculated from the rearranged Bohr equation. Alveolar oxygen tension P_{aO_2} mm Hg was derived from the alveolar air equation assuming arterial CO tension to be equivalent to alveolar CO tension. Diffusing capacity of the lungs for carbon monoxide D_{LCO} ml STPD/min/mm Hg was calculated according

to Filley et al (9) with the correction for CO back pressure suggested by Linderholm (14).

Physical working capacity was determined by a test in the sitting position on a bicycle ergometer according to Sjöstrand (23). The work capacity being expressed as the effect in kpm/min developed on the ergometer at a heart rate of 130 beats/min (W_{130}). Electrocardiograms were recorded by a direct writing ink jet recorder (Minigraph 4, Flema-Schonander Stockholm) at rest in the supine and standing positions during exercise in the sitting position on the bicycle ergometer and $1/2$ and 4 min after exercise. Leads I, II, III, aVR, aVL, aVF, CR₁ and V were used at rest during exercise leads CR₁ with the indifferent electrode on the forehead (22).

The degree of scoliosis was expressed in terms of the angle formed by the converging limb of the spinal curve according to Cobb (8). The severity of the deformation was classified according to Ponseti and Friedmann (19) and the material was divided into two groups: one in which the degree of deformity was from mild to moderate i.e. less than 60° and the other where it was severe $\geq 60^\circ$.

Current statistical methods were used (24).

RESULTS

Anthropometric data on the fifteen scoliotic patients are given in Table I while Tables II and III give the observed and predicted data from the spirometric determinations of lung volumes and ventilatory capacity. The patients' actual height was used for the predictions of lung volumes

Table II Some parametric data on 14 subjects

Mean values (\bar{x}) \pm standard deviation (s) and number of individuals (n) are given

Case no.	VC			TLC			FRC			RV			FRC/TLC			RV/TLC		
	(l BTPS)	(% of predict)	(l BTPS)	(l BTPS)	(% of predict)	(l BTPS)	(l BTPS)	(% of predict)	(l BTPS)	(l BTPS)	(% of predict)	(l BTPS)	(%)	(% of predict)	(%)	(%)	(% of predict)	(%)
1	2.08	63	7.94	1.53	63	1.06	1.53	63	1.06	1.06	63	52	102	52	102	36	100	36
2	1.42	44	3.6	1.67	54	1.24	1.67	65	1.24	1.24	65	63	127	63	127	48	141	48
3	1.55	49	2.19	1.20	59	0.94	1.20	81	0.94	0.94	81	82	108	82	108	42	140	42
4	2.53	93	3.49	1.57	61	0.99	1.57	83	0.99	0.99	83	76	86	76	86	28	89	28
5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6	1.86	64	3.37	1.80	81	1.64	1.80	104	1.64	1.64	104	131	108	131	108	49	163	49
7	1.74	78	4.61	2.63	114	1.87	2.63	129	1.87	1.87	129	145	112	145	112	41	198	41
8	1.38	78	4.50	2.45	93	2.01	2.45	102	2.01	2.01	102	122	106	122	106	44	166	44
9	1.83	76	3.01	1.50	86	1.18	1.50	84	1.18	1.18	84	107	98	107	98	59	130	59
10	1.74	88	4.10	2.81	97	1.84	2.81	124	1.84	1.84	124	119	124	119	124	41	121	41
11	0.68	33	1.33	0.69	49	0.69	0.69	46	0.69	0.69	46	100	102	100	102	49	204	49
12	1.84	72	2.89	1.41	78	1.03	1.41	86	1.03	1.03	86	92	98	92	98	35	120	35
13	2.66	91	4.12	2.89	96	1.46	2.89	96	1.46	1.46	96	103	96	103	96	35	106	35
14	2.56	86	3.96	2.16	88	1.41	2.16	103	1.41	1.41	103	92	108	92	108	36	103	36
15	2.10	78	3.27	3.46	84	1.17	3.46	83	1.17	1.17	83	95	90	95	90	36	116	36
16	1.4	14	1.4	1.4	14	1.4	1.4	14	1.4	1.4	14	14	14	14	14	14	14	14
17	0.6	72.8	3.34	1.77	80.6	1.32	1.77	89.4	1.32	1.32	89.4	100.6	104.8	100.6	104.8	40.0	127.6	40.0
18	± 0.63	± 19.6	± 0.96	± 0.58	± 18.4	± 0.40	± 0.58	± 2.6	± 0.40	± 0.40	± 2.6	± 22.3	± 11.2	± 22.3	± 11.2	± 6.1	± 29.1	± 6.1
Range	0.68-7.4	33-99	1.33-4.61	0.69-3.81	49-114	0.65-2.01	0.69-3.81	46-129	0.65-2.01	0.65-2.01	46-129	63-145	96-135	63-145	96-135	28-49	83-204	28-49

VC = vital capacity; TLC = total lung capacity; FRC = functional residual capacity; RV = residual volume

Table III Ventilatory capacity of 15 subjects

Case no	MVV _F			MVV ₄₀		FEV ₁₀		FEV	
	(l BTPS)	(c/min)	(% of predict)	(l BTPS)	(% of predict)	(l BTPS)	(% of predict)	(%)	(% of predict)
1	57	114	61	39	50	1.43	65	68	92
2	41	76	41	28	34	1.15	48	81	107
3	26	92	27	29	35	0.87	45	73	96
4	47	84	48	31	39	1.65	81	65	87
5	32	109	31	20	24	0.82	39	60	78
6	45	77	43	43	49	1.53	67	8	106
7	66	90	67	45	55	1.75	83	67	88
8	73	94	75	43	52	1.61	66	72	95
9	22	83	23	28	35	1.47	81	82	109
10	45	84	46	47	58	2.03	88	74	99
11	13	67	13	1	14	0.30	18	50	68
12	47	127	58	39	48	1.21	62	68	89
13	83	78	86	69	86	1.84	86	71	95
14	29	80	31	27	34	1.95	90	76	101
15	35	75	36	40	49	1.51	74	73	96
n	15	15	15	15	15	15	15	15	15
\bar{x}	44.7	88	46	36.0	44	1.41	66	71	94
s.d.	±19.7	±15.6	±20.4	±13.5	±16.8	±0.47	±20.8	±8.7	±10.9
Range	13-83	67-127	13-86	12-69	14-86	0.30-2.03	18-90	52-87	68-109

MVV_F, MVV₄₀ = maximum voluntary ventilation at free respiratory rate and at 40 breaths/min. FEV₁₀ = forced expiratory volume in one second. FEV = FEV₁₀ expressed as percentage of vital capacity.

Spirometry

In keeping with previous studies (4, 6, 7, 10, 12, 13, 17) the total and vital capacities—and hence to some extent the functional residual capacity—were smaller than predicted whereas the residual volume was essentially normal. Since the absolute values for the residual volume were its relative measure—expressed as RV/TLC (residual volume/total lung capacity)—was elevated though this is not to be regarded as a sign of obstructive lung disease in the cases under

review. The ventilatory capacities (Table III) that are dependent on both time and volume (MVV_F vs. MVV₄₀ and FEV₁₀) were similarly depressed whereas FEV% (which is independent of the volume) showed ordinary values indicating that the flow was normal.

Certain significant differences in lung volumes and ventilatory capacities were found between the group with moderate scoliosis (10-60°) and the group with a severe deformity (60-102°) of Figs 1 and 2. The former group had higher means for vital capacity and total lung capacity expressed as percentages of the predicted values ($P < 0.01$ and $P < 0.05$ respectively) while RV/

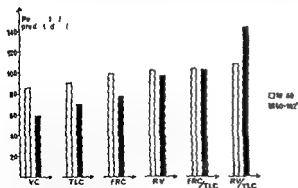


Fig 1 Lung volumes in per cent of predicted value of the two deformity groups. Symbols as in Table II. □ 10-60° ■ 60-102°

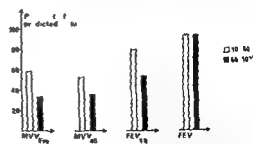


Fig 2 Ventilatory capacities in per cent of predicted value of the two deformity groups. Symbols as in Table III. □ 10-60° ■ 60-102°

TLC was higher for the group with a severe deformity ($P < 0.05$). Similarly the group with a moderate degree of scoliosis had higher means for maximum voluntary ventilation at free respiratory rate and at 40 breaths/min as well as for forced expiratory volume in one second ($P < 0.05$ in each case).

Ventilation and alveolar gas exchange

Data obtained at rest and during exercise are given in Table IV. The load used averaged 86% of the physical working capacity at a heart rate of 130 beats/min (W_{130}) and the pulse rates were 90% of the highest level recorded during the previous test on a bicycle ergometer in the sitting position. No significant differences were found between the two scoliotic groups in the degree of relative circulatory load expressed as percentage of W_{130} or of the highest heart rate recorded previously, but certain differences in the adaptation of ventilatory function were significant.

For the group with a severe deformity the mean total ventilatory minute volume amounted to 72% of the maximum voluntary ventilation (MVV) measured during spirometry whilst the corresponding mean for the group with a moderate deformity amounted to 45% ($P < 0.05$).

The greater ventilatory load for the severe scoliotics is reflected by higher respiratory rates ($P < 0.05$) and smaller tidal volumes ($P < 0.05$) compared with the group with moderate deformities. Shallow rapid breathing usually involves elevated ventilation of dead space with an increased V_D/V_T quotient 0.30 being regarded as the normal upper limit. This value was either reached or exceeded in seven out of fourteen observations during work but there was no difference between the two groups. The ventilatory equivalent for oxygen— V_E/\dot{V}_{O_2} i.e. the ventilation per litre oxygen uptake—amounted to 28 and 27 litres for the groups with severe and moderate deformity respectively, both these values lie within the normal variation.

The alveolar gas exchange was impaired in two cases (nos 3 and 11) in which the diffusion capacity (D_L) and the arterial oxygen tension (P_{aO_2}) were depressed and the arterial carbon dioxide tension (P_{aCO_2}) somewhat elevated indicating a slight carbon dioxide retention. These

cases also had the most severe thoracic deformities 97 and 102 according to Cobb. The corresponding means for the two groups did not differ significantly either from each other or from pertinent normal data.

The alveolar arterial oxygen pressure difference ($P_A - P_{aO_2}$) was higher in most cases than the level of 10–20 mm Hg that most authors regard as the normal upper limit. There was no significant difference between the group means.

The distribution of inhaled gas determined by the nitrogen elimination time was normal in thirteen cases in which it was studied.

The calculated intrapulmonary shunt (Q_{shunt}/Q_{total}) exceeded in many cases 6 to 7% the figure given as the normal upper limit. Assuming that the nitrogen elimination time was normal in the cases in which it was not determined the results indicate that the majority of the fourteen scoliotic patients had elevated shunt values. There was no significant difference between the group means. Case no. 11 in which the diffusion capacity was impaired had a pathologically high P_{aCO_2} during oxygen inhalation the pattern being that of a patient with pulmonary insufficiency who retains carbon dioxide when the hypoxaemic drive on the respiratory centre is eliminated.

DISCUSSION

The value of the present results for assessing cases of idiopathic scoliosis is somewhat curtailed by the positive selection which took place of Nilsson and Lundgren (18). Normal data for the corresponding decades and sex are not available which to some extent makes comparisons uncertain. On the other hand cases with these observations probably have not been published in the literature.

The patients' actual height was used for calculating the predicted spirometric values. It would be wrong to make a correction for the patients' reduced height due to the deformity because as pointed out by Bergofsky et al. (4) it is not possible to estimate the expected normal height for these subjects. It should be considered that in a corresponding female material a difference of 10 cm in height affected the calculation for instance of the vital and the total capacities by 0.41 and 0.67 l respectively. It should also be noted that the use of the actual height

Table IV Ventilation and alveolar gas exchange at rest and during exercise in 14 subjects

Subj no	Load (kpm/min)	Ventilation (l BTPS/min)	Resp rate (c/min)	Tidal vol (l BTPS)	$\frac{V_D}{V_T}$	$\frac{V_E}{V_{O_2}}$	Oxygen uptake (ml STPD/min)	Heart rate (beats/min)
1	Rest		13					118
	300	27.9	24	1.16	0.31	28.6	973	128
2	Rest		39					64
	200	30.0	50	0.60	0.39	37.5	801	118
3	Rest		18					110
	200	31.0	45	0.69	0.37	28.0	1108	155
4	Rest		21					67
	150	22.6	22	1.03	0.38	37.1	609	99
6	Rest		18					—
	300	23.4	24	1.10	0.75	29.3	901	143
7	Rest		16					56
	400	28.5	18	1.58	0.21	26.4	1084	140
8	Rest		—					70
	300	24.4	18	1.36	0.30	26.7	914	114
9	Rest		—					58
	200	21.6	32	0.68	0.22	27.7	779	112
10	Rest		—					68
	200	18.8	27	0.70	0.35	23.9	783	115
11	Rest	3.9	17	0.23	0.25	25.5	154	—
	100	10.5	22	0.48	0.34	19.8	529	—
12	Rest	5.7	15	0.44	0.29	27.0	210	—
	300	26.5	24	1.11	0.23	26.0	1020	144
13	Rest	5.1	17	0.30	0.33	28.8	178	—
	300	27.7	18	1.54	0.23	25.7	1075	—
14	Rest	6.1	19	0.32	0.37	27.4	224	71
	300	21.7	20	1.03	0.14	21.8	996	150
15	Rest	8.0	17	0.47	0.35	33.5	239	72
	300	29.1	23	1.27	0.13	29.9	974	110

V_D = dead space V_T = tidal volume V_E = total ventilation V_{O_2} = oxygen uptake P = pressure a = arterial A = alveolar
 Stbk = standard bicarbonate Q = blood flow

^a After 10 O₂ inhalation ^b After 5 O₂ inhalation

sides involving an underestimation of the calculated volumes also leads to an underestimation of the reductions observed in the lung volumes.

The reduction of the static lung volumes and the ventilatory capacities that depend on volume was more marked for the group with severe orthopedic deformities and indicates a restriction of the lung function. Restriction in this sense includes changes in the lung parenchyma and the chest wall as well as in the respiratory muscles. However the normal absolute values for the residual volumes suggest that the lung tissue itself had not undergone restrictive changes.

On the other hand the abnormal relationship between respiratory volume and vital capacity

indicates an impairment of the chest bellows function. Measurements of work of breathing in a body respirator have been reported by Bergofsky et al (4) who found that when scoliotic patients were ventilated with fractions of the vital capacity similar to those of normal cases the work of the chest bellows was five times as great. The increased work of breathing with the normal quotient between tidal volume and vital capacity indicates that this increment is dependent not only upon the size but also upon the deformity of the thorax. In the present study the relationship between tidal volume during work and vital capacity did not differ between the severe and moderate groups indicating that the patients

$P_{A,O}$ (mm Hg)	P_{CO} (mm Hg)	pH	Stbk (mEq/l)	Diff cap (ml STPD/min) (mm Hg)	$(P_A - P) O_2$ (mm Hg)	During inhalation 100 O_2			
						Nitrogen elimination (time min)	$\frac{Q_{shunt}}{Q_{total}}$	P_{H_2O} (mm Hg)	$P_{A,CO}$ (mm Hg)
79	36	7.43	4						
77	43	7.33	11	14.7	3.4	1.83	0.053	563	29
77	39	7.41	24						
81	39	7.39	22	14.4	25	45	0.065	584	35
74	43	7.42	25						
67	48	7.37	20	9.5	8	2.17	0.059	583	44
111	37	7.46	24						
96	35	7.44	23	16.4	15	2.62	0.043	633	29
80	43	7.37	23						
94	39	7.37	21	17.1	13	1.18	0.061	595	38
86	37	7.46	23						
93	40	7.35	20	26.4	14	3.50	0.063	590	38
89	35	7.43	23						
92	46	7.37	22	32.6	8	1.50	0.091	549	34
85	33	7.40	23						
86	39	7.34	22	14.2	20	2.67	0.070	578	37
98	37	7.44	25						
93	44	7.37	23	24.1	2	1.67	0.038	624	35
68	45	—	—		2.8				
36	46	7.39	—	7.9	26	—	0.101 ^d	5.4	55
67	39	7.43	—		34				
73	38	7.37	—	21.4	33	—	0.103 ^d	528	41
74	38	7.42	25		23				
77	34	7.41	22	19.8	24	—	0.117 ^d	504	37
74	43	7.4	24		14				
111	46	7.37	22	24.0	15	—	0.160 ^d	408	47
81	37	7.45	—		23				
89	35	7.41	—	20.0	17	—	0.107 ^d	510	36

adapt to the increased breathing work. Above a certain limit, as in the two most severely deformed patients, an increased load on the chest bellows in connection with muscular exercise upsets the normal alveolar gas exchange and results in an alveolar hypoventilation with carbon dioxide retention and hypotaemia. The reduced diffusion capacity may thus be a consequence of an alveolar hypoventilation with a "functional" reduction of the surface area for gas exchange.

Previous bronchspirometric studies (25) have shown that gas exchange is impaired in the region of the lung on the concave side of the curvature. The findings of elevated values of alveolar arterial oxygen pressure gradients—as in a disturbed ventilation/perfusion relationship—and of "physiological shunts" are consistent with regional functional disturbances.

The demonstrated relation between the skeletal deformity and the disturbance of lung function agrees with previous reports (4, 6, 17, 20). The functional importance of the skeletal deformity is well illustrated by the finding that the case with the most severe scoliosis (102) developed signs of pulmonary insufficiency during the inhalation of 100 per cent oxygen, emphasizing the clinical significance of making a thorough examination of the lung function in scoliotic patients.

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CEREBRAL BLOOD FLOW AND OXYGEN CONSUMPTION
IN BARBITURATE POISONING

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Abstract Cerebral blood flow (CBF) and cerebral metabolic rate of oxygen (CMR_{O_2}) have been determined by the nitrous oxide method in five cases of severe barbiturate poisoning (blood barbiturate level 2.2-15.4 mg/100 ml). All cases needed prolonged artificial ventilation (IPPV) to survive. Mean CBF was 30 ± 9 ml/100 g min and mean CMR_{O_2} was 1.5 ± 0.4 ml/100 g min. Within the temperature range studied (31.7-37.8°C) body temperature (BT) was correlated to CMR_{O_2} according to the equation $BT = 6.6 \times CMR_{O_2} + 25$; the correlation coefficient being 0.96 ($p < 0.01$). A similar correlation was found between CBF and BT ($r = 0.93$, $p < 0.05$). The cerebral arterio-venous oxygen difference was normal and rather constant in spite of generally low values for arterial PCO_2 . There was no indication of inadequate oxygen supply to the brain.

The mortality rate in barbiturate poisoning has been markedly reduced during the last decade (2, 9, 12, 16). However, owing to the large number of poisonings, certain complications, chiefly pulmonary oedema and persisting cerebral injuries, are seen in many patients. The treatment of these complications may be difficult and the underlying mechanisms are but incompletely known (6, 7).

A barbiturate poisoning is characterized by, among other symptoms, hypothermia. In the most severe cases body temperature descends below 30°C. The barbiturates inhibit certain steps of the respiratory chain in the cells (8, 11), thereby decreasing metabolism and oxygen consumption. In addition, hypothermia reduces the metabolism and oxygen consumption (1, 8). Gleichmann et al. (3) in 1962 demonstrated a connection between depth of narcosis as determined by EEG and reduction in cerebral cortical oxygen consumption in dogs. However, there are as yet only a few investigations reporting data on cerebral blood flow (CBF) and cerebral metabolic rate of oxygen (CMR_{O_2}) in barbiturate poisoning in man. Gottstein et al. (4) determined CBF and CMR_{O_2} in seven patients suffering from

superficial barbiturate induced coma. None of their cases needed artificial ventilation. CMR_{O_2} was reduced by 35-70%. These extremely low values were normalized following administration of analeptic drugs. These authors presume that nerve cells die when oxygen consumption decreases below 20% of the normal value. This is their justification for using central stimulating drugs in the treatment of barbiturate poisoning. However, vast clinical experience has eliminated this kind of drugs from the therapeutic arsenal in Scandinavia (2, 9, 12, 16). Hence it was considered of interest to determine CBF and CMR_{O_2} quantitatively in severe cases of barbiturate poisonings treated according to The Scandinavian Method. Especially the relation between CMR_{O_2} and body temperature was considered to be of interest in order among other reasons to further elucidate the prolonged time of cerebral survival during circulatory arrest and a possible cerebral genesis of the pulmonary oedema. In this preliminary study CBF and CMR_{O_2} have been measured in a small material without the above mentioned complications in order to provide basic values for further investigations in progress.

MATERIAL

The patients were selected for these studies according to the following criteria: (a) Deep coma with hypothermia and need for prolonged artificial ventilation caused by proven barbiturate poisoning. (b) Body temperature slowly increasing at the moment of measurement but not exceeding 38°C. The reason for this limit was that, after passing 38°C, the patients usually return gradually to consciousness. (c) The patients had to be rather stable with respect to body temperature which was not allowed to change more than 0.2°C one hour before and one hour after the measurement of cerebral blood flow. In the original material one patient was excluded because the temperature rose 2.0°C in one hour during and after the measurement. (d) No history or signs

Table I Clinical observations in five patients unconscious due to barbiturate poisoning

Pat no	Age	Sex	Type of barbiturate	Blood conc of barb ^a (mg/100 ml)	Duration of coma (h)	Duration of resp treatm (h)	Lowest temp (°C)	Temp at CBF measurement (°C)	Dose of nor epinephrine (µg/min)
1	37	♂	Comb ^b	12.3	48	34	35.1	37.8	II
2	29	♀	Comb ^b	15.2	52	38	33.9	34.7	12
3	33	♀	Comb ^b	9.0	20	12	34.3	34.3	9
4	59	♀	Shortacting	2.2	70	65	30.7	31.7	6
5	56	♀	Comb ^b	10.0	42	16	33.5	37.2	6

^a Calculated as sodium phenobarbital^b Combination of short and long acting barbiturate

of cardiac or cerebro vascular disease (e) Criteria *a* to *d* had to coincide at a time when clinical and laboratory facilities were available

The relevant data about the five patients and their clinical condition is summarized in Table I. They were deeply unconscious because of attempted suicide with different barbiturates. They were artificially ventilated (IPPV) and received intravenous infusion with Ringer glucose solution about 2000 ml/24 h. All patients on admission had low arterial blood pressure which was effectively treated by norepinephrine administration (6–12 µg/min). The systolic blood pressure was kept constant at 100 to 130 mm of mercury. All the patients had a period of hypothermia. Analyses of barbiturate concentration in blood were made on admission and revealed levels of 2.2–15.2 mg/100 ml blood calculated as phenobarbital. A screening procedure was used to exclude the presence of other poisons in blood and urine. All the patients survived the poisoning without complications.

METHOD

Cerebral blood flow (CBF) and cerebral metabolic rate of oxygen (CMRO₂) were determined by the nitrous oxide method of Kety and Schmidt (5). Arterial blood samples were collected from teflon catheters percutaneously introduced into the brachial or radial artery. The left jugular bulb was

punctured with a hypodermic needle. Five pairs of arterial and jugular venous blood samples were taken at appropriate intervals during a measuring period of 15 min during which 15% of nitrous oxide was added to the inspired air via the Engstrom respirator. Samples for analysis of oxygen content were taken immediately before the administration of the nitrous oxide mixture. Samples for determination of arterial P_{O₂} and P_{CO₂} were taken at intervals before, during and after the CBF measurement. All blood samples were taken in 10 ml siliconized all glass syringes. The dead space in the syringes was filled with 5% heparin solution. The volume of the dead space was determined and the values for nitrous oxide and oxygen content were corrected for dilution by the heparin.

Blood analysis The nitrous oxide content was analysed in duplicate by the method of Orcutt and Waters (10) with the slight modification introduced by Kety. Oxygen content was determined in duplicate by the Van Slyke-Neill manometric technique (17). The oxygen analyses were done immediately after sampling. Arterial oxygen tension (P_{O₂}) and carbon dioxide tension (P_{CO₂}) were measured with a macro oxygen electrode (Beckman) and a Severinghaus electrode respectively, mounted in a Beckman Modular cuvette. The readings were made directly on a Beckman physiological gas analyser after calibration with analysed gas mixtures. The measurements were made at 37°C. The

Table II Cerebral blood flow and oxygen metabolism in five patients deeply unconscious due to barbiturate poisoning

Pat no	CBF (ml/100 g/min)	(A-V) _{O₂} (vol %)	CMRO ₂ (ml/100 g/min)	Temp ^a (°C)	P _a O ₂ ^b (mm Hg)	P _a CO ₂ ^c (mm Hg)	BP ^d (mm Hg)
1	41	4.7	1.9	37.8	60	28	110/90
2	73	6.3	1.4	34.7	55	26	120/90
3	27	6.0	1.6	34.3	89	33	110/85
4	21	4.7	1.0	31.7	73	32	130/90
5	36	4.9	1.8	37.2		25	110/80
Mean ± s.d.	30 ± 9	5.3 ± 0.8	1.5 ± 0.4			29 ± 4	

^a Rectal temperature during CBF measurement^b Arterial P_{O₂} corrected to body temperature^c Arterial P_{CO₂} corrected to body temperature^d Riva Rocci method

values were corrected to the body temperature according to the Severinghaus blood gas calculator (Radiometer)

Statistical methods The statistical calculations were performed according to Snedecor (13). The following probability (p) levels of significance were used: $p < 0.001$ highly significant, $p < 0.01$ significant and $p < 0.05$ probably significant.

RESULTS

The values for CBF, cerebral arterio-venous oxygen difference ($A-V$)_{O₂}, CMR_{O₂}, body temperature at CBF measurement, arterial P_{O_2} , arterial P_{CO_2} and arterial blood pressure are presented in Table II.

From the arterial P_{CO_2} values it can be seen that the patients have been hyperventilated. Mean arterial P_{CO_2} was 29 ± 4 mm Hg. Despite this there were generally low values of arterial P_{O_2} , which is common in unconscious patients being artificially ventilated with air. However, the prevailing oxygen tension corresponded to oxygen saturation values in the range 90–96%, and the oxygen available to the brain was hence satisfactory since the arterial blood pressure was kept constant.

At the time of the CBF measurement three of the patients had hypothermia, the others had normal body temperature.

Cerebral blood flow All cases had values lower than those given by Kety and Schmidt (5) for healthy conscious individuals. Except in case 1 the values may be regarded as very low. In the three cases with hypothermia the values were extremely low. The mean value for the material was 30 ± 9 ml/100 g/min.

Cerebral ($A-V$)_{O₂} was normal or low and rather constant in all five cases; mean value 5.3 ± 0.8 vol %.

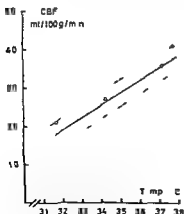


Fig. 1 The relation between CBF and body temperature. The regression line \pm SD is shown. The equation for the regression line is $y = 1.5 - 8.5$, $S.D. = 3.4$, $r = 0.93$ ($p < 0.05$).

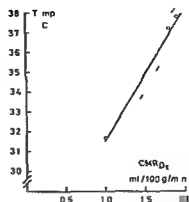


Fig. 2 The relation between body temperature and CMR_{O₂}. The regression line \pm SD is shown. The equation for the regression line is $y = 6.6x + 2.5$, $S.D. = 0.8$, $r = 0.96$ ($p < 0.01$).

Cerebral metabolic rate of oxygen was reduced in all cases. In case 4 the extremely low value of 1.0 ml/100 g/min was found. The mean value was 1.5 ± 0.4 ml/100 g/min.

Correlations The relation between CBF and body temperature is shown in Fig. 1. CBF was probably significantly correlated to body temperature ($r = 0.93$, $p < 0.05$).

The relation between body temperature and CMR_{O₂} is shown in Fig. 2. Body temperature was significantly correlated to CMR_{O₂} ($r = 0.96$, $p < 0.01$). No correlation was found between CBF and arterial P_{CO_2} in these cases.

DISCUSSION

In barbiturate induced deep coma in man there was found a considerable reduction in cerebral oxygen consumption as an expression of the reduced metabolism in the brain tissue. The reduction amounted to about 50% in the whole material. The case with the lowest temperature had a reduction of about 70%. Of special interest is the linear correlation between cerebral oxygen consumption and body temperature ($r = 0.96$, $p < 0.01$). Of course this correlation holds only for the temperature range of the material 31.7–37.8 °C.

The degree of reduction in cerebral oxygen consumption in these cases is higher than the reduction observed during induced hypothermia combined with the general anesthesia. Most authors (1, 8) report a reduction of about 50% for a 10 °C lowering of the body temperature. In the present investiga-

tion there was a reduction of 50% already at a body temperature of about 35°C i.e. two degrees below normal. This big difference between the two conditions is explained by the fact that in the presented material body temperature sunk mainly as a result of reduced metabolism and heat production and not as a result of active cooling.

Bering working with monkeys in induced hypothermia found a linear correlation between $\log CMR_O$ and the reciprocal of the absolute temperature (1). This was true for the temperature range of 15–37°C. A close inspection of his diagrams reveals a near linear correlation between temperature and CMR_O in the range of 32–37°C. Thus the results in man so far agree with those in monkey.

In addition there was found a linear correlation between cerebral blood flow and body temperature which was probably significant ($r=0.93$ $p<0.05$). There was no correlation at all between arterial P_{CO_2} and CBF or the cerebral $(A-V)_O$. The latter was rather constant in all cases despite big differences in arterial P_{CO_2} . These results suggest that cerebral blood flow follows the metabolic demands of the brain tissue despite big variations in arterial P_{CO_2} . Definite conclusions in this respect necessitate further studies.

Judged from the normal or low cerebral $(A-V)_O$ there was no indication of inadequate blood supply to the brain as a whole. However there may be smaller parts of the brain with inadequate oxygen supply in spite of a normal $(A-V)_O$.

The results presented in this work do not support the opinion of Gottstein (4) who recommended the use of analeptic drugs in treatment of these cases. According to him nerve cells die when oxygen consumption is reduced below 20% of the normal but he does not give any reason for his opinion. The lowering of the oxygen demands of the brain ought to be a protection against anoxic brain injuries in these cases. The use of analeptic drugs in treatment of barbiturate poisonings increases the oxygen demand in the nerve cells which may be dangerous in these conditions with reduced cerebral blood flow and disturbed regulation of cerebral circulation.

It is well known that in hypothermia the brain tissue survives circulatory arrest longer than in normothermia. The time interval between the cessation of circulation and the development of irreversible brain injury depends on the degree of oxygenation at the moment of circulatory arrest (15). But

also the rate of cerebral oxygen consumption determines the rate of lowering of the cerebral oxygen tension and thereby the development of brain injury. It is a common clinical impression that the results of cardiac resuscitation are more successful in hypothermic poisonings than in other conditions. In other words the time interval seems to be prolonged. Of course it is not possible to predict how many minutes of cardiac arrest the brain will survive in hypothermic poisonings because we do not know the degree of oxygenation at the moment of cessation of circulation. On the other hand it has been shown that the amount of ATP is increased in nerve cells during barbiturate narcosis (8, 14).

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (Project No. B67 17A 615 0*) and the Stockholm Foundation for Medical Research.

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CORTICOSTEROIDOGENIC EFFECT OF LONG ACTING BETA¹ CORTICOTROPHIN (CIBA 42 915 BA)

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Abstract Synthetic beta¹ corticotrophin, synthesized by Kappeler and Schwyzer has been modified by the manufacturer by adsorbing it onto zinc phosphate. The adrenal cortex action of this preparation has been evaluated in eight non-endocrine patients. A prolonged corticosteroidogenic effect is found as reflected by the increase of plasma corticosteroids (cortisol + corticosterone) and urinary 17 ketogenic steroids. One mg of the modified beta¹ corticotrophin (Ciba 42915 Ba) injected i.m. maintained significantly elevated plasma corticosteroids for at least 36 hours.

Corticotrophin (ACTH) from sheep pig and man is a straight chain polypeptide containing 39 amino acids (4 23 32). Specific species variation is only found in amino acids 25-33 (24).

From the study of fragments obtained by hydrolysis of highly purified natural ACTH as well as by synthesis it can be seen that only the first N terminal 19-24 amino acids are necessary to obtain organotrophic activity of the same range as that of natural ACTH (15 16 25).

In 1961 Kappeler and Schwyzer (19) synthesized beta¹ corticotrophin with an amino acid sequence identical with N terminal 1-24 amino acids in natural ACTH and in 1963 Schwyzer and Sieber (31) reported on the total synthesis of beta corticotrophin with an amino acid sequence characteristic of the porcine species.

In 1962 a third international standard for corticotrophin was introduced (2). The biological activities of synthetic beta¹ corticotrophin and of synthetic porcine beta-corticotrophin have been compared with this standard. Synthetic beta¹ corticotrophin has a biological activity equivalent to 106 units/mg (30). Comparative experiments in man reveal that there is no significant difference in the effect of natural ACTH and of beta¹ corticotrophin on urinary 17 ketogenic steroids (17

KGS) 17 ketosteroids (17 KS) and plasma corticosteroids (18 20 21 22). Synthetic porcine beta corticotrophin has a biological activity equivalent to 115 units/mg (3).

Beta¹ corticotrophin has been used to test adrenocortical responsiveness (1 18 26 33). However since its action is of short duration (22 33) it has little therapeutical use.

The manufacturers have modified beta¹ corticotrophin by adsorbing it onto zinc phosphate. It has been shown that this preparation has a prolonged action (5 13). The present paper reports on the adrenal cortex action of this preparation in eight patients. The preparation (Ciba) will be marketed under the name Synacthen Depot®.

MATERIAL

The material consisted of eight patients (from Diaconisse Institutet, Medical Dept. (Head T Høden MD)). The investigations were carried out while the patients were convalescents. There were no signs of endocrine disorders, kidney or liver diseases. None of the patients had been given or was under treatment with ACTH or adrenal cortex steroids. All patients were of normal weight.

METHODS

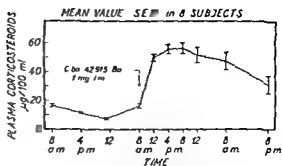
Each patient received 1 m. Ciba 42915 Ba i.m. at 8 a.m. Plasma corticosteroids were estimated at 8 a.m. 4 p.m. and 11 p.m. on the day prior to treatment, immediately before injection and 4 8 12, 16 24 and 36 hours later.

All blood samples were drawn in heparinized glass tubes by venepuncture. Plasma was separated on the same day and stored deep frozen until analysis.

Unconjugated corticosteroids (cortisol + corticosterone) in plasma were determined by a fluorimetric method slightly modified (3) in accordance with de Moor and Steeno (7). 4-hour urine specimens were collected on the day before the day of and the day after injection. 17 KGS were estimated on the 24-hour urine specimens (9).

Table I Plasma corticosteroids and urinary 17 ketosteroids on the day before the day of and the day after i.m. injection of 1 mg Ciba 42915 Ba

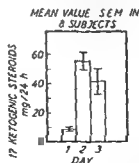
Case no.	Sex	Age (y)	Plasma corticosteroids ($\mu\text{g}/100 \text{ ml}$)										Urinary 17 KGS (mg/24 hour)		
			First day			Second day				Third day			Day 1	Day 2	Day 3
			8 a.m.	4 p.m.	11 p.m.	8 a.m.	12 a.m.	4 p.m.	8 p.m.	12 p.m.	8 a.m.	8 p.m.			
1	+	55	21.5	10.1	8.7	12.2	51.0	57.2	54.8	52.7	41.7	21.3	17.0	76.0	44.5
2	+	75	18.3	9.1	5.8	16.6	49.3	55.8	5.5	62.3	63.4	31.6	5.9	16.4	73.2
3	+	63	13	10.0	8.4	25.7	67.6	74.2	75.5	65.8	39.9	10.0	8.4	55.7	20.7
4	+	49	19.9	10.6	4.5	15.0	40.4	58.2	56.0	57.5	55.7	37.4	4.7	43.1	35.8
5	+	49	12.8	11.7	6.8	15.8	41.8	38.0	38.0	20.8	18.7	9.1	6.2	26.7	9.6
6	+	78	15.1	12.9	3.8	15.1	50.3	49.3	58.5	58.5	71.8	45.8	6.3	43.3	83.3
7	+	73	14.7	13.5	9.7	13.8	49.8	56.0	59.2	58.6	57.9	51.5	6.6	63.0	37.4
8	+	45	14.4	14.8	9.6	15.2	50.8	48.9	49.9	36.0	30.5	31.4	12.0	57.4	27.1
Mean value			16.24	11.34	7.16	15.93	49.50	55.39	56.18	51.56	47.45	31.26	8.39	55.20	41.39
S.E.M.			1.15	0.5	0.81	1.49	2.38	3.23	3.69	5.47	6.31	6.12	1.46	6.03	8.94

Fig. 1 Plasma corticosteroids in eight non-endocrine patients (mean value \pm S.E.M.) on the day before the day of and the day after i.m. injection of 1 mg Ciba 42915 Ba

RESULTS

Table 1 reproduces the values of plasma corticosteroids and urinary 17 KGS on the day before the day of and the day after injection of Ciba 42915 Ba. Mean values and standard error of the mean are given in the table and in Figs 1 and 2.

The statistical significance of the corticosteroidogenic effect of Ciba 42915 Ba has been evaluated by Student's *t* test. A normal diurnal variation in plasma corticosteroids is found on the day before injection. Mean plasma corticosteroids at 8 a.m. on the day before and at 8 a.m. immediately before injection are the same ($P > 0.1$). Four hours after injection mean plasma corticosteroids have increased significantly ($P < 0.001$). Maximum mean values are found 8–12 hours after injection. A slight though not significant ($P > 0.1$) fall in plasma corticosteroids is found 16–24 hours after injection. Thirty-six hours after injection mean plasma corticosteroids have fallen significantly ($0.02 < P < 0.05$) although the mean value is still higher

Fig. 2 Urinary excretion of 17 ketogenic steroids in eight non-endocrine patients (mean value \pm S.E.M.) on the day before the day of and the day after i.m. injection of 1 mg Ciba 42915 Ba

than the mean value of plasma corticosteroids at 4 p.m. on the day before injection ($0.01 < P < 0.02$)

A significant increase in mean 24 hour 17 KGS on the day of injection is found ($P < 0.001$). A slight but not significant ($P > 0.1$) fall in 24 hour 17 KGS is found on the day after injection.

No local reactions except slight tension and tenderness were observed during and for some minutes after the injections.

DISCUSSION

The present experiment clearly shows that the modified synthetic beta¹-corticotrophin (Ciba 42915 Ba) has a prolonged corticosteroidogenic effect as reflected by the increase of plasma corticosteroids (cortisol + corticosterone) and urinary 17 KGS. This is in accordance with the results obtained by Gilboa et al. (13) and Besser et al. (5). In this study maximum levels in plasma corticosteroids are reached 8–12 hours after intramuscular injection. This has also been found by Besser et al. (5). They found a significant clinical effect of 1 mg "depot tetracosactrin" for about 32 hours while this study has demonstrated a significant clinical effect of the same dose for at least 36 hours.

Besser et al. (5) observed no significant difference between 80 units of corticotrophin gel and 1 and 2 mg "depot tetracosactrin" during the first four hours. However the depot tetracosactrin maintained plasma corticosteroid levels longer than corticotrophin gel.

From the results of Besser et al. (5) and from the present study it may be concluded that to maintain continuously elevated plasma corticosteroids 1 mg of the depot preparation of synthetic beta¹-corticotrophin need be given only once daily.

Hypersensitivity reactions in man to ACTH of animal origin has been described on several occasions (7, 12, 14, 29). However unmodified synthetic beta¹-corticotrophin appears to have lower antigenicity. In animal experiments only extremely small quantities of antibodies to beta¹-corticotrophin have been demonstrated (8, 10, 11, 17). It seems evident that the immunogenic activity of natural ACTH is chiefly attached to the amino acids 25–39 (8, 17) which explains the low antigenicity of beta¹-corticotrophin.

In clinical trials to date several patients hyper-

sensitive to natural ACTH preparations have been treated with beta¹-corticotrophin without untoward reactions (6, 18, 20, 22, 33).

The manufacture of depot preparation of synthetic beta¹-corticotrophin has made prolonged treatment with beta¹-corticotrophin possible in man. Experiments with this modified preparation are needed before any firm conclusions can be drawn concerning the antigenicity of beta¹-corticotrophin in man.

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MASTOCYTOSIS TREATED WITH L HYOSCYAMINE (EGAZIL[®])

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Abstract A case of a 38-year-old woman with a mastocytosis syndrome is presented. The urinary excretion of histamine, methylhistamine and 1-methyl-4-imidazole acetic acid was considerably higher than normally. Treatment with L-hyoscyamine sulphate abolished gastritis, diarrhoea and pressure induced pain, and reduced dermographism and itching. However, there was no effect on the abnormal urinary histamine excretion.

The concept of urticaria pigmentosa (up) as a purely dermatological disease has changed to cover the generalized manifestation—systemic mastocytosis (sm) (24). It has also been accepted that sm at least in adult patients carries a certain risk of over morbidity and possibly also some over mortality. Until recently treatment has been rather unsuccessful with the exception of a few patients in whom a histidine decarboxylase inhibitor has been used (18).

This communication reports on the effects of L-hyoscyamine on some symptoms in a patient suffering from sm. Studies on the histamine metabolism were also performed.

CASE REPORT

The patient was a 38-year-old married housewife who was admitted to hospital in 1966 because of abdominal pain, flushing and weight loss. On admission there were three main groups of symptoms.

1 Gastrointestinal symptoms

Since at least 1940 she had had epigastric cramping pain, heartburn and belching with acid taste appearing several times a year mostly in spring and autumn. These symptoms which appeared daily since the spring of 1965 were aggravated by hunger and also after meals, particularly when she ate pears, rosehip soup, marzipan or acetylsalicylic acid. In 1953 a peptic ulcer was verified by X-ray.

Since youth and more since 1961 she usually had diarrhoea once after dinner. In February 1966 after an

upper respiratory infection she had transient diarrhoea 5-6 times a day with voluminous, yellow white faeces with glittering surface floating on water. Since May 1965 she had unvoluntarily lost ten kg of weight.

2 Cutaneous symptoms

The same agents that provoked abdominal discomfort also initiated a cutaneous flush with a feeling of heat mostly on chest and back, sometimes accompanied by itching and often by fatigue, malaise and anxiety. Ever since childhood she always reacted more than usual to scratches, contact with stinging nettles, and to insect bites, with swelling and redness. She had had a brownish erythema since 1948.

3 Pain symptoms

For several years the patient had experienced a deep smarting pain after pressure (e.g. blood pressure determination and embraces) which was most disagreeable. Because of this feeling as of a thousand needles she could not lie still for any length of time especially not on a hard surface.

Several doctors had ascribed her symptoms to nervousness and secondary flushing and abdominal pain in spite of the fact that she had always been careful to point out that the symptoms started with epigastralgia, then flushing appeared and then she became apprehensive.

There were no laboratory findings indicating hyperthyroidism. Intradermal testing with some 40 allergens including her own home dust, was negative.

In March 1966 she was admitted to hospital for further investigation of the abdominal symptoms.

There were few abnormal physical signs. There was no enlargement of lymph nodes in her or spleen. However, over her body and extremities there were numerous millimetre sized, pigmented well delineated slightly infiltrated maculopapules. These appeared more clearly after blunt scratching. In addition there was pronounced dermographism.

Dermatologist's report

The clinical diagnosis was urticaria pigmentosa. Microscopically there was a dense infiltration of mast cells in the upper corium.

not expect the patient to develop such a strong flush and greatly increased epigastric pains during the first day of the treatment. These symptoms reappeared when the patient resumed the treatment after having neglected the tablets for a few days. This transient deterioration could have been due to drug induced histamine liberation. The histamine studies did not show a further increase in urinary excretion of histamine or its metabolites when Egazil treatment was again started. For some reason however the patient did not develop the usual initial flush on this particular occasion.

The mode of action of 1 hyoscyamine in producing the beneficial effects on several symptoms in the present case remains obscure. The possibility of a spontaneous remission seems remote as such remission rarely occur in adults.

The fact that the treatment relieved the gastric symptoms in this patient may or may not be connected with the systemic mastocytosis. It could be regarded as the expected effect of an atropine like drug in any patient suffering from duodenal ulcer.

It might be that the 1 hyoscyamine produced mast cell degranulation and histamine release. This could explain the temporary aggravation of symptoms when treatment was started or was resumed after an interval. The continuous administration of the drug could conceivably produce a continuous liberation of histamine which would keep the stores partially emptied and thereby prevent massive intermittent release. However the measurements of histamine and metabolites in the urine do not provide support for such an action of the drug.

Another tentative explanation might be lowered sensitivity to histamine. The 1 hyoscyamine could have acted as an anti histaminic agent or it could in some indirect way have facilitated the adaptation of the organism to the increased amounts of histamine produced in this patient.

As yet we have observed no untoward effects of the treatment. Theoretically one might speculate that if a major action of atropine is mast cell degranulation this could produce osteosclerosis by liberation of mucopolysaccharides and stimulation of ground substance formation (1). It seems less likely that such degranulation would cause osteoporosis by liberation of heparin which has been shown to induce bone resorption (16).

ACKNOWLEDGEMENTS

This investigation was supported by grants from US Public Health Service No 5 R01 AI03379-08 and from the Swedish Medical Research Council No 46714X 639-03.

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COMPARISON OF RUBELLA HAEMAGGLUTINATION INHIBITING AND NEUTRALIZING ANTIBODY CURVES IN NATURAL INFECTION

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Abstract Considerable differences are shown in rubella haemagglutination inhibiting and neutralizing antibody curves. A rapid development of the haemagglutination inhibiting antibody to maximal titres is found within the first months of illness while the neutralizing antibody reaches maximum as late as 6 to 15 months after infection. Both types of antibody persist at least 3 1/2 years. With respect to serodiagnosis of rubella the importance of obtaining the first serum sample as soon as possible after the appearance of rash is emphasized regardless of the type of antibody sought.

In a preceding publication the development and persistence of neutralizing antibody in human rubella infection were reported (5). The study was based on a series of nine rubella patients bled at intervals from onset of illness over periods from 1 to 3 1/2 years. Since the haemagglutination inhibition (HI) techniques recently described (3, 7) appear to offer certain advantages over the neutralization (NT) test in the serodiagnosis of rubella it was found desirable to have HI tests performed on the same series of sera in order to establish the HI antibody response curve and to compare this with the previous findings pertaining to rubella NT antibody.

MATERIAL AND METHODS

In previous publications detailed descriptions have been given of the human sera employed (5) and of the assay for rubella NT antibody (4).

Human sera were obtained from nine patients with a clinical diagnosis of rubella with rash. Specimens were collected at intervals over a period of 1 1/2 to 15 months with an additional blood sample from six patients 3 1/2 years after infection. All sera employed were heat inactivated at 56 °C for 30 min and stored at minus 20 °C until tested.

NT tests were performed in rabbit cornea (SIRC) cells employing serial twofold dilutions of the sera starting at 1:4. All sera were tested with the addition of a normal

guinea pig serum to the medium used for dilution of sera and virus (4).

HI tests The haemagglutinating rubella antigen used in this study was supplied by Flow Laboratories Inc. Rockville Md USA. The sera were examined in the Takatzy microtitration system employing essentially the technique described by Halonen et al (3). All sera were absorbed with kaolin to remove non specific inhibitors and 0.9% saline made from demineralized water was used as diluent for serum and antigen. Antibody titrations were performed employing twofold serial dilutions starting at 1:10.

NT antibody titres have been calculated by the method of Karber and expressed as the reciprocal of the initial serum dilution in the serum virus inoculum. The HI antibody titres have been expressed as the reciprocal of the highest initial dilution of serum producing complete inhibition of haemagglutination. Throughout this paper NT titres less than 4 and HI titres less than 10 are recorded as 0.

RESULTS

The results are illustrated in Fig. 1 and summarized in Table I. For each patient two sets of antibody titres have been recorded: one set representing the HI antibody and the other set the NT antibody.

HI antibody response curves

From Fig. 1 it will be seen that rubella HI antibody in all patients appears rapidly after the onset of rash (day 0) rising sharply to maximal titres ranging from 160 to 1280 from 8 to 31 days later.

In three patients (A, C and F) there is a gradual decrease in titre from the maximal value while the HI antibody titre in the rest of the sera remains at the maximal level for periods varying up to 5 months forming a plateau on the HI antibody curve before the decrease in titre begins. In all patients rather low titres of 40 to 160 are seen 6 to 12 months after illness and the HI antibody persists at this level throughout the observation period of up to 3 1/2 years.

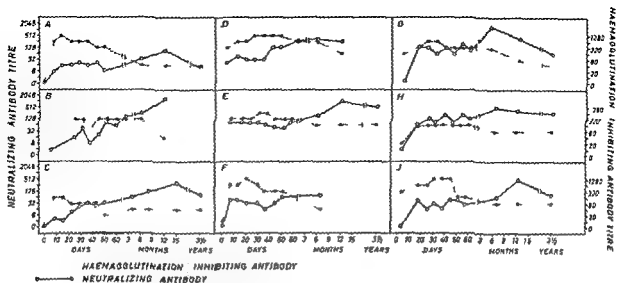


Fig 1 Haemagglutination inhibiting and neutralizing antibody curves for rubella patients A to J Day 0 = onset of rash

NT antibody response curves

The rubella neutralizing antibody curves shown in Fig 1 have been described in detail elsewhere (5). An either gradual or sharp rise in antibody titres is observed within a few days after rash increasing to maximal titres of 90 to 1450 as late as 6 to 15 months after infection. A decrease in titre is seen later although NT antibody still persists 3½ years after illness.

Comparison of HI and NT antibody titres

Table I rubella HI and NT antibody are compared with respect to the time when antibody is

first demonstrated and to the time of appearance of maximal titres.

It will be seen that HI antibody is demonstrable earlier than NT antibody in sera F, G and J while the two forms of antibody can be demonstrated simultaneously in the rest of the patients. A marked rise in the HI antibody titre is found after the first few days of illness reaching levels only two to eightfold lower than the maximum. In one patient (C) the maximal HI titre is found as early as 8 days after rash and in all patients peak HI titres are reached within the first month of illness. In contrast the first demonstrable NT

Table 1 Comparison of rubella haemagglutination inhibiting (HI) and neutralizing (NT) antibody titres in individual sera A-J

Pat	Antibody first demonstrated				Maximal titre				Titre 3½ years after illness	
	Day		Titre		Day		Titre			
	HI	NT	HI	NT	HI	NT	HI	NT	HI	NT
A	7	7	640	8	14	376	1280	90	40	15
B	6	6	80	4	25	378	320	1450		
C	8	8	160	6	8	457	160	360	40	90
D	4	4	120	23	27	182	1280	360		
E	7	7	320	90	31	367	640	1074	160	51
F	1	7	10	45	11	02	180	90		
G	5	18	160	18	25	161	640	1450	40	64
H	4	4	0	4	17	193	160	517	80	360
J	4	18	370	45	31	370	180	51	80	90

* Patient followed for 20½ days only

antibody titres are low in one patient (B) several hundred fold lower than the maximal NT titre and the highest NT titres are not found until 6 to 15 months after illness. Common to the two forms of antibody are relatively low titres in the 3 1/2 year specimens.

DISCUSSION

The serological diagnosis of virus infections is normally accomplished by examination of paired serum specimens collected from the patient 2 to 3 weeks apart early in illness.

In a previous report (5) we stressed the necessity of collecting the first serum sample in rubella infection at the appearance of rash or immediately thereafter in order to be able to demonstrate a fourfold or greater rise in neutralizing (NT) antibody titre. The findings of the present study suggest that prompt collection of the acute phase serum sample is even more important when the serological rubella diagnosis is based on the haemagglutination inhibition (HI) test since the HI antibody appears earlier or at least simultaneously with the NT antibody. Thus in three of the nine patients studied HI antibody was found in acute phase sera in which NT antibody was not yet demonstrable. If serum samples collected during the very first days after rash had been available from all patients the number of sera demonstrating HI antibody in the absence of NT antibody might have been even greater.

If HI antibody is already demonstrable in the first blood specimen collected from a rubella suspect patient the possibility of a serodiagnosis based on the HI test will almost entirely depend on the proper spacing of the paired serum samples. Occasionally however the steep initial rise in HI antibody titres such as seen in four patients of this study may permit the demonstration of a significant titre rise even when the paired sera are collected with only a short interval. If the first serum sample is not collected promptly at the onset of rash the present findings show that the generally employed interval of two weeks between blood samples is not always sufficient for a significant HI antibody titre rise to develop. Further it will be seen that after the first appearance of HI antibody a fourfold titre rise may in most cases only be demonstrable if blood samples from the maximal titre period can be included in the test.

The average time of appearance of maximal titres for the patients examined was day 22 with individual variations from day 8 to day 31. This finding suggests that in order to demonstrate a rise in HI antibody titre it may be necessary to collect blood specimens at weekly intervals until four weeks after the onset of rash. It should be pointed out however that even if this is done a serodiagnosis based on HI antibody determinations may not always be feasible. Thus it has not been possible to show a fourfold or greater rise in HI titre in the sera collected from patient E and the same would have applied to patients A and C if an early antibody negative serum sample had not been available.

Rubella HI titres have been reported to be considerably higher than the corresponding NT antibody titres (2, 6, 7). From the data in Fig. 1 it is seen that HI as well as NT antibody titres of individual sera are highly dependent on the time of collection. Thus if a comparison is made of the HI and NT antibody titres when they are first demonstrable Table I shows that eight of the nine patients studied have higher HI than NT titres while a comparison of the maximal titres reveals that only four of the nine patients show higher HI than NT antibodies. In the serum samples collected 3 1/2 years after illness only one of the six patients from whom sera are available shows an HI antibody titre which is higher than the corresponding NT titre. The difference in the course of the HI and the NT antibody response curves apparently reflects the demonstration of different antibodies rather than a higher sensitivity of one test or the other.

It is generally accepted that the protective value of serum products is dependent on the contents of neutralizing antibody. Our findings are therefore of considerable practical importance in relation to serum products intended for rubella prophylaxis. These should not be selected on the basis of a high rubella HI titre since in many instances this is not correlated to a high NT antibody titre. On the basis of the present study it is suggested that "convalescent serum" for passive rubella immunization should be derived from blood donors 6 to 15 months after clinical illness when the neutralizing antibodies have reached their maximum and not as is now common routine 3 to 6 weeks after onset of rash.

Since in the present study HI and NT antibody

could be demonstrated simultaneously in all sera collected after the first few days of illness and be cause—as stated in a recent review by Dudgeon (1)—it appears that even very low levels of neutralizing antibody are indicative of immunity to rubella it seems permissible to use the HI test to determine the status of naturally acquired immunity to rubella

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HYDROXOCOBALAMIN

Excretion and Retention of Repeated Large Doses in Patients with Pernicious Anaemia

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Abstract The retention of hydroxocobalamin was found to be approximately the same after each of four 48 hourly injections of 1 mg of hydroxocobalamin in untreated pernicious anaemia. After four such injections a maximum of 2-3 mg of the vitamin is retained corresponding to approximately 50% of the normal stores.

The retention of vitamin B₁₂ after one injection is much higher for hydroxocobalamin (OH B₁₂) than for cyanocobalamin (CN B₁₂). OH B₁₂ is thus more effective for replenishing B₁₂ stores in a state of deficiency. The normal amount of stored vitamin B₁₂ has been calculated to be about 4 mg (3). After an injection of 1 mg which is the dose most commonly used, 50-70% is retained (1, 2, 4, 6). This means that the restitution of the stores if completely empty will take some 8-10 injections. Nothing is known however about the in-

terval necessary between the injections. It might be surmised that too frequent injections could result in progressively less retention due to saturation of the primary binding sites of the vitamin. The present study was undertaken to find out the retention after four 48 hourly injections of 1 mg of OH B₁₂. This regimen was chosen for practical reasons since most patients with untreated pernicious anaemia do not need hospital care for more than 8 to 10 days.

MATERIAL AND METHODS

Sixteen patients with untreated pernicious anaemia were given by injection 1 mg of hydroxocobalamin every second day for eight days i.e. a total of 4 mg. 24-hour urines were collected, the volume measured and frozen aliquots stored until assayed. Great care was taken not

Table 1 24 hour urinary excretion of vitamin B₁₂ following injections of 1 mg OH B₁₂ on days 1, 3, 5 and 7

Case no	Day								Total excretion (μg)	Calculated retention (mg)
	1	3	4	5	6	7	8			
1	47	3	121	16	166	44	233	76	706	3.3
2	322	4	318	13		17	251	25		
3	191	23	304	116	247	155	263	34	1330	2.6
4	781	34	215	80	285	112	378	60	1375	2.6
5	203	747	346	76	71	137	272	68	1570	2.9
6	416	86	459	137	312	75	235	57	1777	2.2
7	163	18	110	52	156	49	201	52	801	3.2
8	639	87	182	48	161	46	242	51	1501	2.5
9	171	17	218	30	267	78	289	63	1128	2.8
10	186	36	175	76	202	118	66	102	961	3.0
11	87	19	121	59	128	56	138	58	666	3.3
12	70	35	200	53	98	50	156	64	726	3
13	60	18	208	31	233	54	146	77	1027	3.0
14	14	14	160	30	188	56	02	65	857	3.1
15	157	12	157	16	238	53	308	63	1004	3.0
16	285	50	375	92	419	114	461	150	1896	1

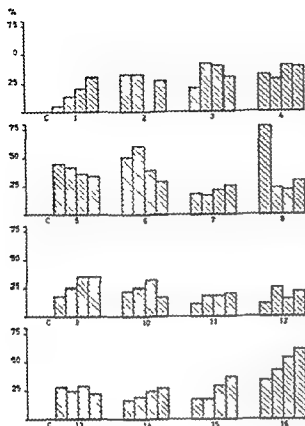


Fig 1 48 hourly urinary excretion of B_{12} after injection of 1 mg OH B_{12} four times on alternate days to 16 patients

to leave the urine samples at room temperature. The vitamin B_{12} activity was estimated microbiologically according to Hutner et al (5).

RESULTS

All urinary excretion values for vitamin B_{12} are presented in Table I together with the calculated retention values. These were found to be in the range of 2.1–3.3 mg with a mean value of 2.8 mg. The 48 hour urinary excretion results (i.e. the time between injections) are shown in Fig 1.

The fifth to sixth day urine from case 2 was incompletely collected and is thus not included in the table or the figure. As seen in Fig 1 three cases 1, 15 and 16 and perhaps also case 9 show a continuous increase in urinary excretion. In the other ten cases there is no such obvious tendency suggesting a rather equal retention after each injection.

The first 48 hour urinary excretion of case 8 was much higher (77%) than any of the other excretion values. The reason for this is obscure.

DISCUSSION

Whitey and Kilpatrick (8) compared the effect of repeated 48 hourly doses of 1 mg of CN B_{12} and OH B_{12} on the serum B_{12} levels in untreated cases of pernicious anaemia. In one of their figures it can be seen that there was no significant difference between the urinary excretion after the first and fourth injection in two patients. This agrees with the results that we have found and to our knowledge this is the only other observation published of a similar nature.

The present study shows that the calculated retention of OH B_{12} after injections of 1 mg repeated every 48 hours is the same even after the fourth injection. This kind of treatment will thus provide an amount of the vitamin approximately equalling 50–75% of an estimated normal B_{12} store of 4 mg. Of course it must be borne in mind that these retention figures are probably somewhat too high. There is a small excretion on the third and fourth day after an injection usually amounting to a few per cent or less. Further the retention values are based on excretion figures which may be too low due to methodological shortcomings (7).

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INTESTINAL CLOSTRIDIUM PERFRINGENS IN RHEUMATOID ARTHRITIS AND OTHER COLLAGEN DISEASES¹

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Abstract The intestinal anaerobic flora has been studied with special reference to *Clostridium perfringens* in various collagen diseases: rheumatoid arthritis (RA) in particular and in a series of control cases. The *C. perfringens* flora was qualitatively and quantitatively abnormal in two thirds of 186 RA cases and in two thirds of enteroarthritis cases in about half the SLE, the psoriatic arthritis and the seronegative polyarthritis cases, but only in a few of the patients with pelvispondylitis ossificans and uroarthritis (Reiter's syndrome). An abnormal *C. perfringens* flora was not demonstrated in any of the patients with polyarthritis or arthralgia after tonsillitis nor in any of the control cases. The alpha anti-toxin titre in serum was raised in 78% of the RA cases, and such titre rises which occurred parallel to the pathology. *C. perfringens* flora were also demonstrated in the cases of other inflammatory rheumatic diseases but were not noted in any of the control cases.

The results of faeces cultures and serology at examinations are analysed in relation to several clinical and laboratory parameters, such as the patient's age and sex, duration and activity of the disease, administered drugs, hypergammaglobulinaemia and secretion of hydrochloric acid in the stomach. A tendency to higher frequency of abnormal *C. perfringens* flora is noted both in RA patients with high disease activity and in elderly patients.

In a previous paper (22) we reported briefly our observations relating to the intestinal *Clostridium perfringens* flora in rheumatoid arthritis and other collagen diseases and to the occurrence of both circulating and cell bound antibodies against *C. perfringens* alpha toxin in some of these diseases. Here we present a more detailed report of our findings in an enlarged material and the methods used. The relation of the findings to various clinical and laboratory parameters as well as their possible pathogenetic significance will also be discussed.

Paper presented at the Vth European Rheumatology Congress October 1967, Lisbon.

MATERIAL

The series comprises 381 patients with inflammatory rheumatic diseases who were treated at the Department of Rheumatology, Karolinska sjukhuset or attended the hospital's outpatient department and 70 control cases.

Rheumatoid arthritis (RA)

186 patients. All fit Ropes et al. (30) criteria for a diagnosis of classical or definite RA. The haemagglutination reaction with sensitized sheep-cells according to the technique of Svartz-Schlossman, was positive in 181 cases. The remaining five patients had either shown a positive reaction earlier or undergone several courses of gold treatment, or had classical erosive arthritis with ulnar deviation without signs of psoriasis, SLE or ulcerative colitis.

Seronegative polyarthritis

45 patients forming a heterogeneous group including patients with probably incipient RA (with bilateral arthritis of small joints often developing in association with upper respiratory tract infection) or polyarthritis involving mainly large joints in whom there was no evidence of SLE, arthropathy, psoriasis, pelvispondylitis, urological disease (Reiter's syndrome) or intestinal disorders of ulcerative colitis or regional-enteritis type and in whom rheumatoid or antinuclear factors could not be demonstrated.

Arthritis or arthralgia after tonsillitis

11 patients. All had histories of throat infection with findings of group A streptococci in cultures of pharyngeal secretion and/or raised antistreptolysin O titre in serum.

Uroarthritis

16 patients in whom the diagnosis was based on Olhagen's (26) criteria. This group includes cases of post gonorrhoeal arthritis and of Reiter's syndrome.

Enteroarthritis

17 patients, whose joint trouble started in direct association with diarrhoeal disease (enteritis or colitis).

Table I. *Intestinal C. perfringens* flora

Diagnosis	No. of cases	Qualitatively ^a and/or quantitatively ^b abnormal		Dubiously ^c abnormal		Normal	
		No.	%	No.	%	No.	%
Rheumatoid arthritis	186	125	67.1	15	8.1	46	24.8
Enter-arthritis	12	11	67	0	—	4	33
Systemic lupus erythematosus	27	16	59.3	4	14.8	7	25.9
Psoarthritis	23	12	52.2	4	17.4	7	30.4
Seronegative polyarthritis	45	21	46.7	5	11.1	19	42.2
PeI = spondylitis ossificans	21	5	23.8	2	9.5	14	66.7
Uro-arthritis (Reiter's syndrome + postgonorrhoeal arthritis)	16	3	18.7	1	6.3	12	75.0
Arthritis or arthralgia after tonsillitis	18	0	—	5	27.8	13	72.2
Controls	70	0	—	7	10.0	63	90.0

^a Qualitatively abnormal: Nagler zones > 5 mm diam.^b Quantitatively abnormal: 10^5 bacteria per gram faeces.^c Dubiously abnormal: Nagler zones 4–5 diam. and/or $> 10^3$ to $< 10^5$ bacteria per gram faeces.*Peliospondylitis ossificans*

21 patients in whom X-ray examinations showed findings consistent with bilateral sacroiliac joint arthritis and paravertebral syndesmophytes according to Romanus et Ydens (9) radiographic criteria.

Arthropathia psoriatica

23 patients who had typical psoriatic lesions together with erosive arthritis of characteristic localisation and in whom tests for rheumatoid factor were negative.

Systemic lupus erythematosus (SLE)

27 patients with arthritis or arthralgia or a history of these disorders and evidence of present or previous visceral manifestations raised ESR and in most cases hypergammaglobulinaemia more than a few LE cells and/or high titres of antinuclear factors.

Control series

46 healthy volunteers belonging to the hospital personnel and 74 arthrosis patients age range 18–79 years.

METHODS

A. Examination of Faeces

General comment

Patients who had been treated with sulphadiazine or antibiotics less than two weeks before the sampling were not included. As most of the patients had an active disease some had taken antirheumatic drugs during the sampling period: mostly moderate amounts of acetylsalicylic acid 1–3 g daily. Some of the RA patients had received prednisolone about 5 mg daily (or an equivalent dose of some other steroid). About one-third of the patients had also had a chloroquine preparation 200–250 mg daily. The figures shown in Table I refer to

the results of the first examination at admission to the hospital irrespective of whether samples taken later were positive. The outpatients brought with them faeces samples at their first visit to the rheumatological department.

Bacteriological examination of faecal samples

5 g of a fresh faecal sample stored at +4°C for at most 4 hours were transferred to a flask. Physiological saline solution was added to make a total volume of 30 ml and homogenisation was done by careful stirring. If not used for culture immediately the material was kept at –20°C.

Comparative studies using a dilution fluid other than physiological saline such as a buffer solution, peptone water etc. have shown that the physiological saline solution has no inhibitory effect on the *C. perfringens* flora. Equally freezing to –20°C has not decimated the *C. perfringens* flora (but has had this effect on other groups of microbes such as coliform bacteria).

The number of *C. perfringens* was determined by tenfold dilutions as follows. To a series of tubes was added 4.5 ml of physiological saline, 0.5 ml of the material to be examined was transferred in the first tube. After change of pipette the content of this tube was mixed and 0.5 ml was then transferred to the next tube and so forth. At the same time 1 ml of each dilution was transferred to a series of tubes containing 1% lactose broth and 1 ml to each of three series of Petri dishes. To the first of these was added 5% horse blood, 2% to the second, the same medium, and to the third 5% egg yolk agar. The two latter plate series were incubated anaerobically (hydrogen gas environment) and the others aerobically. Cultures were made at 37°C for 10–12 hours. The number of *C. perfringens* was determined by counting the colonies in egg yolk agar and blood agar. The count was made on two successive plates in the series. The diameters of the precipitation zones in egg yolk agar indicating the lecithinase production were measured. The

Table II Serum levels of *C. perfringens* alpha-antitoxin

Diagnosis	No of cases	Titre (0.001-0.01 U/ml)		Titre (>0.01-0.10 U/ml)	
		No	No	No	No
Rheumatoid arthritis	160	31	21.9	175	78.1
Enterio-arthritis	7	1		1	
Systemic lupus erythematosus	25	10	40.0	15	60.0
Psoriatic arthropathy	15	7	46.7	8	53.3
Seronegative polyarthritis	38	19	50.0	19	50.0
Pelvic spondylitis ossificans	20	13	65.0	7	35.0
Uro-arthritis	15	12	80.0	3	19.8
Arthritis or arthralgia after tonsillitis	13	12	92.3	1	7.5
Controls	50	50	100	0	—

strains which were typed in ordinary way are to be designated as *C. perfringens* A more detailed description of certain properties common to strains belonging to the different groups of the material studied will be given later on (74)

B Serologic Analysis

Determination of the alpha-antitoxin titre in blood serum

Serum was inactivated at 50°C for 30 minutes. Dried filtrate of strain 11607C (*C. perfringens* Wellcome Research Laboratories) which lacks a detectable amount of theta toxin was used as test toxin (alpha toxin). The dilution fluid was calcium gelatin saline (Cagsal) 138.75 ml of 1% CaCl₂ solution 100 ml of 5% gelatin physiological saline 27.5 g of NaCl and distilled water to 250 ml. Six fresh sheep-erythrocyte suspension was used as indicator (the sheep cells were washed three times).

The serum samples were tested in small tubes (Dreyer's tubes). Equal amounts of serum were tested against various dilutions of the test toxin (T/100 T/100 T/300 T/400 T=2 units of alpha toxin per ml). The content was mixed and the tubes were allowed to stand at room temperature for 30 minutes. The same volume of sheep-cell suspension (as that of serum and test toxin) was then added. After mixing the tubes were heated to 37°C in a water bath for 60 minutes kept at +4°C overnight and read.

RESULTS

The results of the faeces cultivations are shown in Table I. A qualitatively abnormal flora refers here only to the size of the precipitation zone in egg yolk agar (Nagler zone more than 5 mm in diameter) doubtfully abnormal refers to Nagler zones 4-5 mm in diameter. A quantitatively abnormal flora refers to 10³ *C. perfringens* bacteria per g of faeces or more. Doubtfully abnormal means >10³ and <10⁴ bacteria per g of faeces. It will be seen from Table I that up to now we have not found Nagler zones larger than 5 mm

in the control cases that two-thirds of the RA patients had a definitely abnormal flora and that the few enterioarthritis patients had the same high frequency of a pathological *C. perfringens* flora. Next in order are the SLE psoriatic arthritis and seronegative polyarthritis patients of whom 59%, 52% and 47% respectively showed an abnormal flora. On the other hand among the post-infectious joint reactions there is no patient with an abnormal flora in the group of arthritis after tonsillitis. In the uro-arthritis group there are three patients with an abnormal flora including one with chronic Reiter's disease and recurrent diarrhoea. Of the five pelvispondylitis patients with an abnormal *C. perfringens* flora three had intestinal disorders manifested by diarrhoea.

The result of the serological examination set out in Table II shows a marked parallelism with the result of the faeces cultures insofar as a pathological *C. perfringens* flora is accompanied by raised alpha-antitoxin titre. In rheumatoid arthritis the frequency of pathological serum reactions is slightly higher than that of an abnormal *C. perfringens* flora.

This discrepancy may be due to several causes. One of these is that the diet or occasional drugs might have inhibited the growth temporarily. Another cause is that in patients with low counts and high lecithinase activity the demonstration of a few bacteria will be due to chance. Longitudinal studies have shown seemingly inexplicable fluctuations in the faecal *C. perfringens* flora, which have also been observed by earlier investigators in studies of intestinal clostridia in other situations. However we have not found

Table III Frequency of abnormal *C. perfringens* flora in relation to patient's sex in the rheumatoid arthritis group

	No. of patients		
	Total	Men	Women
Abnormal flora	186 67.1	61 70.5	124 66.2

any correlation between abnormal *C. perfringens* flora and altered intestinal motility represented by frequent stools or constipation

Relations of the findings to diverse variables

The result of the faecal cultivations and serum examinations were analysed with respect to a number of variables of possible significance. The large RA group was used as the test object in these analyses. The sex distribution shows the expected preponderance for women (Table III). The frequency of an abnormal *C. perfringens* flora was about the same in both sexes, however. A comparison between the frequency of positive findings in the faeces and raised serum antitoxin titres respectively, shows a rising tendency for higher ages as far as the occurrence of an abnormal faecal flora is concerned (Table IV). In the control series there are 16 arthrosis patients in the age group 50-79 years, none of whom had an abnormal *C. perfringens* flora. One explanation of the higher frequency of an abnormal *C. perfringens* flora with increasing age might be that a longer duration of disease can be expected in these categories. An analysis of the frequency of an abnormal *C. perfringens* flora and raised

Table V Frequency of abnormal *C. perfringens* flora and of raised alpha-antitoxin titre in serum in relation to duration of the disease (rheumatoid arthritis)

Duration	Abnormal flora		Raised alpha antitoxin titre (>0.01-0.1 U/ml)	
	No. of cases	(%)	No. of cases	(%)
3 mo	3		2/3	
3-12 mo	10/14	7.1	8/12	
1-5 y	75/47	59	27/36	75
5-10 y	37/48	67	31/38	82
10 y	56/79	71	57/71	80
Total	155/186	67	155/180	86

antitoxin titres respectively in relation to the duration of illness (Table V) shows however no overrepresentation for a longer period of illness. But the number of patients with a short disease course (less than 1 year) is too small to allow any conclusions as regards this part of the material.

The frequency of an abnormal *C. perfringens* flora in relation to the ESR as indicator of the disease activity (6) shows a tendency to increase with increasing ESR as will be seen from Table VI. 93% of the RA patients with an ESR of 100 mm or more in one hour had an abnormal *C. perfringens* flora. The serum antitoxin response shows no such correlation, however. The series includes a few patients with low ESR and virtually inactive disease in whom all the same an abnormal *C. perfringens* flora could be demonstrated.

The rheumatoid arthritis series was analysed for the possible effect of some drugs on the *C.*

Table IV Frequency of abnormal *C. perfringens* flora and of raised alpha-antitoxin titre in serum in relation to patient's age (rheumatoid arthritis)

Age group (y)	186		160	
	Abnormal flora		Raised alpha antitoxin titre	
	No. of cases	of the group	No. of cases	of the group
10-19	1/4		2/3	
20-29	3/10		4/6	
30-39	7/14	50	9/14	64
40-49	3/36	64	26/31	84
50-59	37/5	70	41/46	89
60-69	40/57	77	41/41	71
70-79	14/18	78	14/18	78

perfringens flora (Table VII). No essential differences were noted for aspirin or small doses of prednisolone but it will be seen from the table that the RA patients with a normal flora were to a somewhat greater extent treated with chloroquine preparations. This therapy may thus possibly have led to slightly too low figures for the number of patients with an abnormal flora. However 33 RA patients with an abnormal flora had no drug treatment at the time of sampling.

A measure of the progress of the disease is the occurrence of erosions in bones demonstrated by X-ray. Eighty of the RA patients had such erosions. An abnormal *C. perfringens* flora could be demonstrated in 70.7% of these patients as against 67.1% in the patients without erosive arthritis that is no significant difference. The question whether or not the presence of achlorhydria would be of any significance to the *C. perfringens* flora was studied by means of the Diagnex test in 100 inpatients with chronic arthritis (Table VIII). An abnormal *C. perfringens* flora was noted in 62.7% of the Diagnex positive patients and in 61% of those with a negative test or a weak reaction thus there was no difference.

An analysis for the relation of an abnormal *C. perfringens* flora to ordinary rheumatological parameters such as rheumatoid factor, anti-nuclear factors, antistreptolysin titres and antistaphylococcal titres provided no evidence of such a relationship. Both an abnormal *C. perfringens* flora and raised antitoxin titres occurred for instance in patients with negative rheumatoid factor test as in most of the SLE patients in all the psoriatic arthropathy and all the enteric arthritis patients. Nor was the antitoxin titre correlated with hypergammaglobulinaemia (here defined as a relative gammaglobulin concentration of more than 20% on paper-electrophoretic serum analysis). Of the RA patients 33.4% had hypergammaglobulinaemia according to this definition 66% of these showed an abnormal *C. perfringens* flora and 78% had raised antitoxin titres as against 67% and 78% respectively of the whole RA group thus no deviation from the RA group as a whole. As regards SLE 22 out of 27 patients had hypergammaglobulinaemia 12 having raised antitoxin titres while three had such rises of titre and normal gammaglobulin values among them two nephrosis patients. There

Table VI Frequency of abnormal *C. perfringens* flora and of raised alpha-antitoxin titre in relation to ESR as indicator of disease activity in the rheumatoid arthritis group

ESR (mm/h)	Abnormal flora		Raised alpha antitoxin titre	
	No. of cases	(%)	No. of cases	(%)
< 15	13/22	59	16/19	84
15-50	47/77	61	50/64	78
51-99	51/72	71	47/61	77
≥ 100	14/15	93	12/16	75
Total	125/186	67	125/160	78

Table VII Frequency of drug treatment in relation to abnormal and normal *C. perfringens* flora in rheumatoid arthritis cases

Type of drug treatment/d	Patient groups	
	Abnormal flora treated	Normal flora treated
Acetylsalicylic acid preparations (1-3 g)	65	57
Prednisolone (3-7.5 mg)	29	13
Chloroquine preparations (0.25 g)	30	41

Table VIII Gastric secretion of acid studied by Diagnex test in relation to frequency of abnormal *C. perfringens* flora in 100 consecutive cases of chronic arthritis

	No. of cases	Abnormal flora (%)
Diagnex test positive	51	62.7
Diagnex test negative or weak reaction	49	61.3

Tubeless gastric analysis by means of estimation of azu resin in the urine after ingestion of this dye compound (Diagnex Blue) and caffeine stimulation

was no exclusive correlation between the presence of joint symptoms and an abnormal *C. perfringens* flora or raised antitoxin titres in SLE. Two patients with lupus nephritis alone had an abnormal *C. perfringens* flora in faeces and raised antitoxin titre respectively.

DISCUSSION

Clostridium perfringens belongs to the normal intestinal flora and occurs mainly in the colon. Sporadically it has also been isolated in healthy

persons in samples aspirated through an intestinal tube both from the jejunum and the ileum (14). In comparison with the other anaerobes—*Lactobacilli*, *Bacteroides* and *Fusiformes* which are the predominating organisms in the intestine (8)—the *Clostridia* form a minority (10). Probably because of technical difficulties of cultivation the part played by intestinal *Clostridia* in the production of disease has attracted little interest in comparison with the studies of the aerobic intestinal bacteria. The *Clostridium* flora has been studied mainly in connection with local disorders of intestinal function such as food poisoning, diarrhoea in infants and fermentative dyspepsia. However, at the beginning of the 20th century quite a number of studies were made of the intestinal flora in pernicious anaemia in which it was found that invariably *C. perfringens* occurred in greatly increased amounts in the intestinal contents (11). Of particular interest here is Kahn's (13) study in 1924 of the anaerobic flora in man with special reference to some conditions of then unknown aetiology, notably pernicious anaemia but also obscure diarrhoeas and chronic eczema. Kahn also investigated six cases of chronic arthritis. All six had greater amounts of spore bearing anaerobes in the faeces than had the control cases. All had *C. perfringens* and three had also *C. sporogenes*. Kahn rejected the idea that these bacteria would be of pathogenetic significance; no serological or immunological studies were made. Månsson and Colldahl (21) studied the faecal *Clostridium* flora in patients with bronchial asthma and found a significant increase of the number of *C. perfringens*. In preliminary studies they also found an increase of the *C. perfringens* flora in rheumatoid arthritis; no immunological studies were made.

In the investigation presented here it has been shown that particularly in rheumatoid arthritis but also in several other inflammatory rheumatic diseases the intestinal *C. perfringens* flora is significantly increased and that it has undergone a qualitative change. The strains isolated by us are mostly characterized by high lecithinase activity (alpha toxin production) readily demonstrable by the Nagler effect in egg yolk medium but also by some other properties which seem to distinguish them from *Clostridia* both in patients with achylia and in asthmatics, namely their heat sensitivity and fermentative ability vis à vis inulin (24).

A new observation is the finding of an immunological response of the host organism manifested by circulating antibodies demonstrated with alpha toxin as test substance. Cell bound antibodies have also been demonstrated by skin testing with a purified alpha toxin revealing in virtually all the RA patients a positive allergic reaction of delayed hypersensitivity type (22, 27).

The reported observations give rise to the question whether the findings would have any pathogenetic implications in the collagen diseases. That intestinal disorders can play a part in the causation of joint disease was noted as early as 1672 by Sydenham who pointed out that patients with dysentery can sometimes develop arthritis. In the 19th century Ruhr rheumatismus (dysenteric rheumatism) was an established concept which assumed a new dimension when Reiter (28) and Fiessinger and Leroy (9) observed that in the late course of dysentery there sometimes appeared a triad of symptoms referable to the urinary tract, eyes and joints. So-called colitis arthritis appears in 10–20% of patients with ulcerative colitis (39) and regional enteritis can also be complicated by arthritis involving mainly the sacroiliac joints and the spine. The rare Whipple's disease illustrates that a clinically silent intestinal affection of probably bacterial origin (7) can give rise to joint symptoms several decades before the intestinal symptoms appear. It has also been proposed that rheumatoid arthritis might be of enteric aetiology. In the 1910s–20s a popular concept among some workers was that intestinal autointoxication originating from the upper part of the colon was the cause of rheumatoid arthritis. These ideas led to for instance drastic sometimes apparently successful attempts at treating RA by colectomy (15, 36). Courses of diet directed against putrefactive bacteria in the intestine were also recommended (4). Shatin (32, 33, 34) advocates that RA is a disease of intestinal aetiology. Excessive consumption of gluten containing food, particularly rye and wheat products would according to Shatin lead in malabsorption with secondary amino-acid deficiency.

The first specific evidence for the theory that intestinal *Clostridia* can elicit a peripheral immunological reaction in the host was presented in veterinary medicine by Månsson (16, 17, 18, 19, 20) in his studies on parakeratosis in pigs. Månsson found that the development of par-

keratosis was directly related to an increase of *C. perfringens* in the intestine and to the influence of the antigens of these microbes on the host as evidenced by raised antitoxin titre in serum, elevated ESR and hypergammaglobulinaemia. Mansson et al. (23, 25) in experiments with a high protein diet in pigs were able to show that the parakeratosis is preceded by arthritis involving the interphalangeal joints and accompanied by hypergammaglobulinaemia and raised antitoxin titre in serum.

A fact of more than historical interest is that Achalme (1) in a fatal case of "acute articular rheumatism" with carditis recovered from heart blood, cardiac valves and pericardium anaerobic bacteria with the same properties as those later attributed to *C. perfringens* by Welch and Fraenkel. *Bacillus d'Achalme* the name also given to this *perfringens* species at that time was isolated from both blood and joint fluid in acute polyarthritis by several investigators in the 1890s and the early 20th century (2, 3, 12, 31, 37, 38). Up to World War I many workers believed that it was the cause of rheumatic fever. Bosc and Carrieu (5) showed however that spores of *B. welchii* can be isolated in great numbers from the skin of man and from these investigations they concluded that *Bacillus d'Achalme* is a banal saprophyte which had contaminated the cultures from blood or other material. Simonds (35) also questioned the validity of the concept that *B. d'Achalme* would be the cause of acute articular rheumatism because unlike the findings in gas gangrene autopsy of arthritis cases had not shown any foamy organs.

We look upon the findings of an abnormal *C. perfringens* flora as evidence of a change in the intestinal habitat. We have started investigations with the object of finding out the origin of this disturbance partly by analysis of various conditions attended by functional changes in the glands of the digestive tract. As was mentioned earlier the presence or absence of hydrochloric acid in gastric juice does not seem to play any decisive part. Preliminary studies suggest that equally reduced salivary secretion as in chronic sialoadenitis does not lead to a change in the *C. perfringens* flora. Investigations into the part played by diet and the influence of antibiotics are also in progress.

ACKNOWLEDGEMENTS

These investigations were supported by the Swedish Medical Research Council Project no K 68 16 X 1003-03 and the King Gustaf V 80-year Fund.

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BENCE JONES PROTEINURIA IN BENIGN MONOCLONAL GAMMAPATHIES

Incidence and Characteristics

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Abstract The possibility and the extent to which detection of Bence Jones proteinuria might be used as a clinical differential criterion between benign and malignant monoclonal gammopathies has been investigated. Clinical and laboratory studies were carried out in 42 subjects with an "essential" serum M-component and by comparison in 46 patients with myelomatosis and in ten with macroglobulinemia Waldenström.

The heat precipitation test was found weakly positive in only one urine sample from the benign cases (2.3%) as compared with 32.6% of the myeloma cases and 20% of those with macroglobulinemia. However by combining agarose-electrophoresis and immunoelectrophoresis of concentrated urine a urinary light chain component was demonstrated in 23.8% of the benign gammopathies. For myelomatosis and primary macroglobulinemia the corresponding figures were 67.3% and 60% respectively.

The amount of Bence Jones proteinuria never exceeded 60 mg/l in the benign forms whereas in the malignant forms it changed considerably from case to case and could reach values of as much as 10 g/l.

Wide variations of pH and temperature were studied for their effect upon the thermal behaviour of two kappa type Bence Jones proteins isolated from the urines of two subjects with benign monoclonal gammopathies. With the findings investigated the physico-chemical properties of these benign type Bence Jones proteins did not differ apparently from those described as characteristic of Bence Jones protein in myelomatosis.

The kappa/lambda ratio of the serum M-components in the essential cases did not show any preferential synthesis as compared with myelomatosis and normal immunoglobulins. This indicates that the benign monoclonal mutation of kappa and lambda synthesizing cells may develop at a rate proportional to their normal occurrence.

Now that the electrophoretic and immunoelectrophoretic techniques have become routine proce-

dures in many laboratories detection of serum and/or urine pathological proteins or M-components is no longer a rare occurrence.

Initially considered as pathognomonic of myelomatosis and macroglobulinemia Waldenström M-components have been subsequently found in a large group of patients with different diseases (2, 8, 9, 18, 25, 27, 28, 30, 39) and in apparently healthy subjects as well (1, 10, 15). The condition of an M-component outside plasma cell myeloma and related malignancies has been termed "essential benign monoclonal gammopathy" by one of the present authors (JW) (34-37) and occurs much more frequently than the corresponding malignant forms (1, 16).

Recently the prognostic and diagnostic problems connected with the benign monoclonal gammopathies have been extensively reviewed (16). Among the differential criteria against myelomatosis (usually low remarkably constant levels of the serum M component, absence of skeletal involvement and of diffuse plasmacytosis, follow up studies showing status quo etc.) the absence of Bence Jones proteinuria at least to any marked degree has been emphasized. However the possible excretion of such microglobulins in amounts slightly exceeding the normal level has not been sufficiently elucidated in these forms and it still remains unsolved as to whether and to what extent the demonstration of Bence Jones proteinuria is compatible with a diagnosis of benign monoclonal gammopathy.

In the present study clinical and laboratory investigations were undertaken to ascertain the incidence of Bence Jones proteinuria in a group of benign forms as compared with another group

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Table I *Number, sex and age of the cases studied*

No. of cases	Sex		Age	
	♂	♀	Mean	Range
<i>Benign monoclonal gammopathies</i>				
4	19	23	66.2	32-91
<i>Myelomatosis</i>				
46	25	21	65.5	47-94
<i>Macroglobulinemia Waldenström</i>				
10	6	4	67.4	49-8

of overt myelomas and macroglobulinemias. The light chain typing of M components in both groups was also reported.

MATERIAL AND METHODS

Benign monoclonal gammopathies

Forty-two subjects belonging to this group were examined. All of them had been included in Hallén's thesis (16) and satisfied the selection criteria listed in that paper. The follow-up period ranged from two years and 11 months to 13 years, and all subjects had been after-examined in the spring of 1965.

Malignant forms

Forty-six patients had myelomatosis and ten had primary macroglobulinemia. Diagnoses were established on the basis of the common clinical, radiological and cytological criteria. The patients had been followed up by one of the authors (J.W.) and were last after-examined at the time of serum and urine collection. They were almost all on Alkeran® treatment.

In Table I sex and age (mean and range) of both groups are given.

With the exception of the two cases of IgD-myelomatosis of which only frozen sera and urines were available in the remaining cases fresh serum samples and urines were obtained in the period Oct. 1967–Feb. 1968.

Protein characterization

Serum was separated in two tubes and stored at -70°C . 24-hour urine output was filtered through a double thickness of Whatman no. 1 filter paper in a cool room, and 100 ml samples were allowed to concentrate about 200-fold in the cold Colloidum shells (Membranfiltergesellschaft, Göttingen, Germany) connected to a vacuum pump were used throughout.

Electrophoresis was carried out on $0.5 \times 110 \times 1$ mm glass plates covered with 1% agarose in barbital buffer pH 8.6, 0.75 M. Ten different samples could be separated at one time on each plate and serum and concentrated urine from the same patient were always run in parallel to allow inspection comparison. After the staining with Amido Black 10B the patterns were scanned with a Vitatron automatic densitometer.

Whenever the Heller test (stratification of urine over concentrated nitric acid) was positive the urinary protein was precipitated by 20% trichloroacetic acid. Total protein determinations in both sera and redissolved urinary precipitates were done by the biuret method.

If the electrophoretic pattern of the concentrated urine showed only a discrete component the amount of Bence Jones proteinuria was arbitrarily assumed to be equal to the total urinary protein content. When there was commonly the case other protein fractions were clearly recognizable the electropherogram of the concentrated urine was scanned and the excretion of Bence Jones protein was then calculated as percentage of the total protein content.

Immunoelectrophoresis was performed by the micro-modification of Scheidegger (29) as described elsewhere (7). Total horse antihuman antiserum and strictly specific anti IgG, anti IgA and anti IgM rabbit antisera were obtained from the Netherlands Red Cross Blood Transfusion Service, Amsterdam. Anti kappa and anti lambda rabbit antisera were prepared in this laboratory by intramuscular injection of kappa or lambda chains respectively isolated from the urines of two patients with myelomatosis. The schedule of immunization and the procedures of absorption were as described by Fahey and McLaughlin (12).

Kappa and lambda antigenic determinants of the M components in sera and when present of free light chains in concentrated urines were studied by immunoelectrophoresis. For IgM-components which did not penetrate the agar the sera were tested after dilution incubation with 0.1 M cysteine or increasing the ionic strength of the buffer. In some cases isolation of the M-component was achieved by gel filtration on Sephadex G 100 of 1 ml serum eluted by phosphate buffered saline.

The heat precipitation test for Bence Jones protein was performed in all urine samples in the manner described by Putnam et al. (16). The urines were always acidified by the addition of 2 M acetate buffer pH 4.9 and the tubes were then incubated in a water bath (Ultra Thermostat, Lauda Messer, Lauda, West Germany) at 56°C for 30 min. Afterwards the tubes which showed any precipitation or opacity were heated at boiling temperature for 3 min in the same apparatus and inspected for possible decrease of the precipitate or clearing of the opacity. Temperature and pH precipitation curves (6) were recorded for two purified urinary Bence Jones proteins isolated from two cases of benign monoclonal gammopathies.

Isolation of Bence Jones proteins (for the preparation of antisera and the study of their physico-chemical characteristics) was carried out by 70% ammonium sulphate saturation of individual 24-hour urine samples. After stirring for six hours at room temperature the mixture was allowed to stand overnight at $+4^{\circ}\text{C}$. The precipitate recovered by centrifugation, was washed thrice with a 70% solution of ammonium sulphate and then suspended in a small amount of sodium bicarbonate 0.1 M. The salts were finally removed by exhaustive dialysis against running tap water. Further purification of Bence Jones proteins was obtained by anionic chromatography on DEAE Sephadex A 40 using a gradient elution with 0.1 to 0.30 M phosphate buffer pH 8.

Table V Light chain types of serum M components

Total kappa/lambda ratio	GammaG			GammaA			L-chain (Gamma μ)			GammaD			GammaM		
	K	L	K/L	K	L	K/L	K	L	K/L	K	L	K/L	K	L	K/L
Benign monoclonal gammopathies															
14	23	11	2.0	2	6	0.3	—	—	—	—	—	—	—	—	—
Myelomatosis															
10	17	7	2.4	4	11	0.3	3	2	1.5	0	2	—	—	—	—
Macroglobulinemia Waldenström															
23	—	—	—	—	—	—	—	—	—	—	—	—	7	3	2.3

nous anemia was diagnosed and appropriate treatment started. Since then R.B.C. have always been about 4 mil.

At routine examination in 1960 she appeared in a fairly good general condition. ESR 38 mm/h, M-component 2.4 g/100 ml and gamma fraction 0.7 g/100 ml. Bone marrow plasma cells were 5% and X-ray was negative.

In 1961 she was treated for bronchopneumonia. Plasma cells and gamma fraction were unvaried, the M-component was 2.2 g/100 ml.

The findings were mostly unchanged in 1964 (M-component 2.4 g/100 ml, gamma fraction 0.8 g/100 ml, plasma cells 9%) at the after-examination in the spring of 1965 (M-component 2.6 g/100 ml, gamma fraction 0.8 g/100 ml, plasma cells 3%) and in 1967 (M-component 2.5 g/100 ml, gamma fraction 0.7 g/100 ml).

At the last after-examination in Feb. 1968, after the discovery of a urinary light chain component, the serum M-component was 3.0 g/100 ml and the gamma fraction 0.7 g/100 ml. Plasma cells were 5% and skeletal X-ray was negative. Physical examination showed good general conditions.

Light chain typing

Serum M components and when present urinary Bence Jones proteins were studied in all cases for their kappa or lambda antigenic determinants. The purpose of this investigation was to establish whether or not M-components of the benign type exhibit a preferential light chain type.

In Table V the frequencies of kappa and lambda antigenic determinants both in the benign and malignant forms are given. It can be seen that in the benign monoclonal gammopathies the total kappa/lambda ratio does not differ very much from that found in myelomatosis. And notwithstanding the rather small number of cases examined the differences are not statistically significant even when the kappa/lambda ratios calculated for each immunological type are compared.

DISCUSSION

The occurrence of light chains in the urines from normal adults has long been known (13, 32). These normal free light chains are not derived from the degradation of gamma globulin but represent anabolic product of gamma globulin metabolism (14, 31, 33). The level has been calculated in 2.5 mg of 1 chains/l urine (3) and hence remarkable concentrations of large urine samples are necessary for detecting these normal light chains.

The main purpose of the present investigation was to assess whether and to what extent detection of a Bence Jones proteinuria in amounts exceeding the normal level could be used as a differential diagnostic criterion between benign monoclonal gammopathies and myelomatosis or macroglobulinemia. Indeed, rather few and somewhat contrasting results are reported in the literature on this subject. Hallen (16) found a light chain component in the urine in four out of 90 subjects with benign monoclonal gammopathies. In one of them myelomatosis could not be excluded and in another a prostatic cancer with skeletal metastases was detected at necropsy. There were no signs of myelomatosis. Hobbs (18) found no case of benign M-component with Bence Jones proteinuria in excess of 1 mg/100 ml. Danon et al. (9) observed a positive heat precipitation test in two out of 66 such cases but in 30 of the subjects with low proteinuria urinary immunoglobulin abnormalities could also be demonstrated.

In the present material fresh serum and urine samples were examined in all but two cases of IgD myelomatosis. Moreover, the same techniques were used throughout so as to ensure compar-

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⁶ Identification numbers are the same as used by Hallén (16)

Clinical studies

Although all the subjects included in this investigation had been selected on the basis of a reasonably sure diagnosis (16) the cases of benign monoclonal gammopathies in which Bence Jones proteinuria had been demonstrated were again submitted to a thorough after examination. It included physical examination, sternal puncture, skeletal X ray (skull, ribs, thoracic and lumbar spine, pelvis and proximal parts of the femora), determinations of hemoglobin, red and white blood cells, thrombocytes, blood urea nitrogen and serum creatinine. In two out of ten positive cases the after-examination was only partial because the patients could not or would not co-operate.

A clinical and laboratory summary of the findings in these ten cases is shown in Table IV. The data do not differ significantly from the corresponding values obtained for the same subjects at after-examination in the spring of 1965 (16).

The case history of patient no. 5 (see Table IV) under clinical observation for over ten years will be reported in some detail because of a moderate increase of the serum M-component in the last year.

Case 5

Female born in 1889. She had been largely healthy until Nov 1946 when she was admitted with symptoms of cholecystitis apparently without gallstones. RBC were 33 mill and ESR 18 mm/1 hr. Serum electrophoresis disclosed an M-component (IgG type 1.5 g/100 ml) the concentration of the gamma fraction was 0.75 g/100 ml. X-ray showed no osteolytic lesions.

In Oct 1948 she complained of tiredness, shivering, weight loss. RBC were 2.7 mill and W.B.C 400. The M-component was 1.8 g/100 ml and the gamma fraction 0.7 g/100 ml. Sternal puncture revealed 5+ plasma cells and some megaloblasts. Serum vitamin B was low. Per

Table V *Light chain types of serum M components*

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23	—	—	—	—	—	—	—	—	—	—	—	—	7	3	2.3

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At routine examination in 1960 she appeared in a fairly good general condition. ESR 58 mm/h. M-component 2.4 g/100 ml and gamma fraction 0.7 g/100 ml. Bone marrow plasma cells were 5% and X-ray was negative.

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In the present material fresh serum and urine samples were examined in all but two cases of IgD myelomatosis. Moreover the same techniques were used throughout so as to ensure compar-

able results. It was found that the heat precipitation test is very seldom positive in the benign forms (one case out of 42). However by combining agarose electrophoresis and immunoelectrophoresis of concentrated urines Bence Jones proteinuria was unequivocally demonstrated in 23 of the benign monoclonal gammopathies. Although the percentages of positivity were considerably higher in the malignant forms neither a positive thermoprecipitation test nor electrophoretic and/or immunoelectrophoretic abnormalities of concentrated urine could be demonstrated in about 33% of patients with myelomatosis and in 40% of those with primary macroglobulinemia.

The calculated amounts of Bence Jones proteinuria never exceeded 60 mg/l in the benign forms whereas in myelomatosis the quantities varied remarkably from case to case and could be as high as 10 g/l. Therefore the results suggest that Bence Jones proteinuria of more than 60 mg/l strongly argues for myelomatosis or macroglobulinemia. Conversely the association of a serum M component in a rather low concentration and a urinary light chain component of less than 60 mg/l is not sufficient to warrant a diagnosis of malignancy.

The occurrence of Bence Jones proteinuria outside myelomatosis and primary macroglobulinemia has been repeatedly reported in patients with malignant tumors and skeletal metastases (6, 16, 19, 22). In our subjects with benign monoclonal gammopathies and a urinary light chain component thorough clinical and laboratory examinations were carried out after the discovery of the urinary abnormalities. Nevertheless not only could myelomatosis or macroglobulinemia be excluded in all cases but also malignant tumours with skeletal metastases were not apparently found.

The possibility that an individual case of essential paraproteinemia may represent myelomatosis or macroglobulinemia Waldenström in an early stage cannot be excluded and has actually been proved in isolated instances (5, 20, 24). But on the whole such a possibility seems to be of rare occurrence (16, 35).

Whether or not all or most of the benign monoclonal gammopathies with a urinary light chain component will eventually develop myeloma is a problem which cannot be solved at the present time. Although the observation time was in no case shorter than three years and ranged up to

almost twelve years it is obvious that only further follow up will allow definite conclusions. However it should be emphasized that with the only exception of case no. 5 (Table IV) in which a moderate increase of the serum M-component was demonstrated in the other cases no clinical or laboratory findings suggested as compared with the after examination in the spring of 1965 (16) an evolution process towards malignancy.

Wide variations of pH and temperature were tested for their effect upon the thermal behaviour of two kappa type Bence Jones proteins isolated from the urines of two subjects with benign monoclonal gammopathy. The results suggested that within the limits investigated the physico-chemical properties of Bence Jones proteins from benign forms do not differ apparently from those described as characteristic of Bence Jones proteins in myelomatosis (26).

The frequency of kappa and lambda chains in a large number of serum M-components has been recently studied by Laurell and Snigurowicz (21) and by Wollheim and Snigurowicz (38). However no clinical distinction between benign and malignant forms was made by these authors. On the other hand Hammack (17) claimed a preferential synthesis of kappa type light chains (20 cases out of 20) in a group of "idiopathic paraproteinemias". Our present results in agreement with those of Danon et al. (9) would seem to suggest that the total kappa/lambda ratio of serum M-components in the benign forms does not differ significantly from the ratios found in myelomatosis and in normal immunoglobulins (4, 11, 23). Therefore it seems reasonable to assume that the cells which synthesize kappa or lambda type immunoglobulins may undergo a benign monoclonal proliferation with a frequency roughly proportional to their normal occurrence.

ACKNOWLEDGEMENT

This work was supported in part by Svenska Livnäkningssocieteten namnd for medicinsk forskning.

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COMPLICATIONS IN MEASLES WITH SPECIAL REFERENCE TO ENCEPHALITIS

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Abstract In the 15 year period 1948-1962 4874 patients were hospitalised in the City and County of Copenhagen with measles. All the case reports have been studied concerning complications and mortality rate special attention being paid to the incidence and the prognosis of encephalitis. The patients surviving from encephalitis have been followed up.

The most severe complications were as expected pneumonia and encephalitis. Pneumonia was found in 1.6% patients, 12 of these patients died. Encephalitis was found in 68 cases among which six deaths. Before the new therapeutic principles dating from the great polio epidemic in 1952 five of 21 patients died from encephalitis, but after that time only one of 47 patients died. Of the survivors from encephalitis 59 (95%) were followed up 3-16 years after the disease, three suffered from grave defects, 16 had mild defects and 40 had recovered completely.

The frequency of encephalitis was 0.43% of the notified cases of measles in the period of investigation. Among the admitted patients 2% died (4.5%). The mortality rate in the notified cases was only 0.14%.

Encephalitis is a rare complication in measles and concerning mortality rate and sequelae is not so severe as considered previously.

Vaccination against measles has been used on a large scale in many countries in the last 6-7 years. In Denmark vaccination has been used only in some districts in Greenland (4). Before entering into a discussion whether to vaccinate the whole population or not it is of course desirable to know how severe the disease is and especially the incidence of encephalitis and the sequelae of this complication.

In this paper complications other than encephalitis will be only briefly mentioned where after encephalitis and its sequelae are assessed after a follow up examination of the survivors will be discussed in detail.

MATERIAL

In the 15 year period 1948-1962 4874 patients (616 males and 2258 females) with measles were admitted to hospitals in the City and County of Copenhagen, of whom 4730 to the Department for Contagious Diseases, Blegdamshospitalet and 144 developed measles during their stay in the pediatric departments in the City and County of Copenhagen (the pediatric departments of Rueshospitalet, Blegdamshospitalet, Sundby Hospital and Amtssygehuset Gentofte and the Dronning Louise and Fuglebakken pediatric hospitals). In the region as a whole 157 300 cases of measles were notified during the same period. As 298 700 children were born in this region in 1947-1961 the numbers of notified cases are not remarkably small.

The curves comparing the numbers of hospitalised cases with the numbers of notified cases in the eight epidemics in the period are seen to concur (Fig. 1). Most of the hospitalised patients were in the age-group 1-4 years (Table I) which does not mean that the disease is most common in this age-group as it is to be expected that the incidence of hospitalisation of patients with measles falls with increasing age. In the age-group 4-5 months the disease does occur but relatively rarely. In the age-group 6-11 months measles is as common as in children above 12 months of age as for comparison, a compensation must be made for the shorter time span of the younger group. A relatively large group were more than 20 years old; this group includes 98 patients from Greenland who contracted measles during their stay in Copenhagen.

All the case reports were studied by the author herself.

RESULTS

Complications

Complications were seen in 2470 (51%) of the admitted 4874 patients with measles. In 305 (6%) cases two or more complications occurred. In 1585 (32%) patients there were no complications. In the remaining 17% the patients were

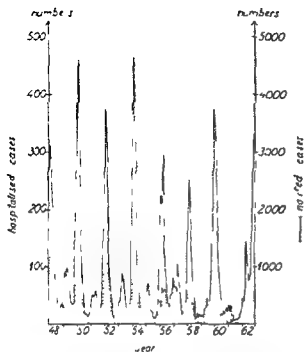


Fig 1 Measles. Comparison between admitted and notified cases. The numbers of cases are drawn from month to month. In the eight epidemics during the period the two curves are seen to concur.

admitted because of other diseases and measles appeared during their stay in the hospital. The complications catarrhal otitis media, acute bronchitis and acute enteritis will not be discussed.

Pneumonia was a frequent complication being found in 1268 (26.0%) cases (Fig 2). From the case reports it was impossible to say whether the cases were primary measles pneumonia or bacterial pneumonia. The relative annual incidence was the same during the period of investigation (Fig 3). Twelve patients (0.9%) died from pneumonia. Bacteriological diagnoses were only made in five of these cases; in two cases *Staphylococcus aureus* was found.

Suppurative otitis media was found in 861 patients (17.7%) but complicating osteitis only in three cases. In two of the cases purulent meningitis developed without sequelae.

Acute laryngitis was found as a complication in 213 patients (4.1%); 154 males and 59 females, whereas in no other complication was found a sex difference. Ten of these patients were tracheostomised; all of them were decannulated within ten days.

Lymphocytic meningitis was found in 58 patients (1.2%). In none of these cases did the case history or the course of the disease cause suspicion of encephalitis.

Purulent meningitis was found in 13 cases (0.3%) of these one case was fatal (meningococci); the other patients were discharged without sequelae.

Encephalitis

Among the hospitalised patients encephalitis was found in 68 (1.4%); 35 males and 33 females, all but one between the ages of six months and 10 $\frac{1}{2}$ years, the average age being 5 years. A single patient was 52 years old. The annual number of cases within the period of investigation varied from zero to eleven (Fig 4).

The diagnosis encephalitis in this publication based on the clinical picture. In most of the cases encephalitis started with loss of or clouding of consciousness. Convulsions alone were not regarded as a sign of encephalitis, nor were transitory reflex changes in relation to convulsive fits without concurrent paralysis.

The cerebral symptoms began from two to fourteen days (mean five days) after the onset of the rash, and in 90% with loss or clouding of consciousness (Fig 5). The spinal fluid was examined in 67 of the 68 cases. Pleocytosis was found in 50 cases. Quantitative determination of

Table I Age incidence among 4874 patients admitted with measles

Three of the patients were under four months of age, none of their mothers had had measles. Among 2-4 patients over 0 years of age 93 were Greenlanders who contracted measles during their stay in Copenhagen.

Age (y)	No of cases	Per cent of total
1*	476	8.7 ^b
1-4	3203	65.9
4-9	854	17.5
10-14	89	1.8
15-19	78	1.6
> 20	24	0.6
Total	4874	

0-3 mo 3 patients 4-5 mo 34 patients 6-11 mo 389 patients

* The figure 8.7% in children under one year of age is only comparable with the percentages for the other groups when compensation has been made for the time span of the younger group.

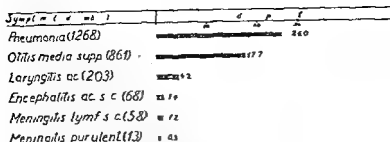


Fig. 4 Complications among 4874 patients admitted with measles. In 297 (6.1%) two or more of the complications mentioned were found. In 1585 (32.3%) no other disease than measles. Besides these complications, catarrhal otitis

media, acute bronchitis and acute symptomatic enteritis were found. Altogether 7470 (50.7%) of the admitted patients with measles had complications.

protein in the spinal fluid was done in the cases admitted after 1951. Among 43 cases 20 patients had spinal protein above 40 mg per 100 ml. Electroencephalography was performed only in 12 patients.

Besides the symptoms mentioned in Fig. 5 mutism was found in one case together with motor disturbances. In another case the only symptoms were sudden deafness and motor disturbances.

Follow up examination

In the acute stage of encephalitis six of 68 patients died; all of the 62 primary survivors are known to be still alive. The follow up examination of these patients comprises 59 (95%). Forty five patients appeared for follow up examination while another 14 answered an enquiry by letter. The observation period ranged from three to sixteen years, being over five years for 50 (85%) of the examined patients.

All the examinations were carried out by the author. During the interview with the patients interest was concentrated on the patients intelligence, how they had managed at school and whether behavioural changes had been noticed. Attention was also focused on possible disturbances of gait and reflexes. Neither spinal fluid examination nor ophthalmoscopy or electroencephalography were performed.

From the results of the follow up examination it is seen (Table II) that 40 patients (68%) had recovered completely while three suffered from grave defects. Of these one had cerebral palsy and two were very feeble minded, one of whom had already been suspected of being mentally retarded before the measles.

From the table it is seen that six of the patients were mildly backward, of whom two were known to have managed well at school before the encephalitis, having severe difficulties at school after the disease. As regards the other four patients it is not possible to say whether their backwardness resulted from the disease or whether they had been mentally retarded previously.

Behavioural changes were met with in two patients. Besides these two patients one patient

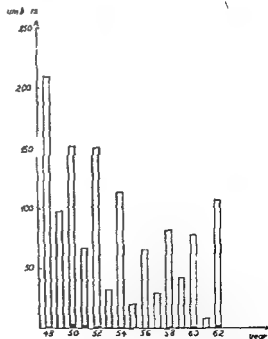


Fig. 3 Pneumonia, total 169. The relative annual number of cases was almost the same during the period of an estimation. The black columns show the fatal cases.

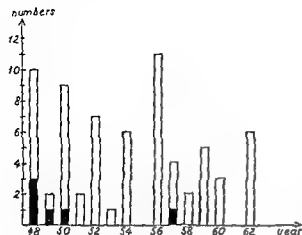


Fig 4 Encephalitis total ■ The annual number of cases varied from zero to eleven The black columns show the fatal cases

was found to have anxiety neurosis perhaps resulting from a knowledge of the encephalitis contracted 11 years previously

Epilepsy (petit mal) was found in one patient in whom the symptoms began four years after the encephalitis but this epilepsy well controlled no longer caused her any trouble as she had had no symptoms during eight years of treatment Grand mal was found in one of the two feeble minded patients

The patient who became deaf still had impaired hearing but managed well with a hearing aid

Headache was reported in two patients neither of whom used analgetics

The remaining three patients had mild and rather dubious sequelae One patient (now aged 18) still had enuresis 14 years after the disease before the encephalitis she had no troubles of

this kind The remaining two patients (aged 14 and 16) had slight walking troubles neither reflex changes nor muscular weakness were found at the follow up examination

One boy had greatly impaired muscular strength in the legs and mutism but six months later he had recovered completely and was found normal at the follow up investigation three years later

As regards all patients there seems to have been proportionality between the period with clouding of consciousness and the liability to sequelae it seemed justifiable to exclude sequelae due to hypoxia

Fatal cases

Among the 68 patients with encephalitis six died their age being from six months to seven years These six deaths were distributed as follows during the first three years of the period of examination five deaths occurred among 21 patients in all while during the last twelve years only one of 47 patients died

Among the first five fatal cases two died of respiratory failure two during prolonged convulsive fits and one in hyperthermia during a complicating pneumonia The last of the six patients died from hemorrhage from the innominate artery after tracheostomy and intubation Autopsy was performed in all but one of the six cases Microscopic examination of the brain showed encephalitis

Of the admitted 4874 patients 22 died (4.5% of the admitted patients 0.14% of the notified 157 300 cases) Among the 1268 patients with pneumonia 12 (0.9%) died The frequency of death from pneumonia is nearly the same at the

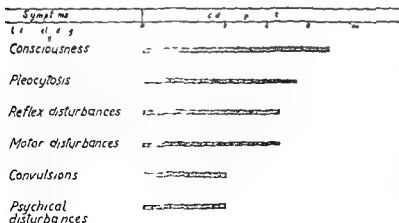


Fig 5 Incidence of symptoms in encephalitis Convulsions alone were not regarded as signs of encephalitis nor were transitory reflex changes in relation to convulsive fits without concurrent paralysis Quantitative determination of protein in spinal fluid was only performed in patients admitted after 1951 in these 43 patients spinal protein was above 40 mg in 6 cases

Table II Follow up examination

Among 59 (95 %) of the 62 survivors grave defects were found in three patients mild defects in 16 while 40 (68 %) had recovered completely from their encephalitis

Normal	40
Cerebral palsy	1
Feeble minded	2
Backwardness	6
Behavioural changes	2
Anxiety neurosis	1
Epilepsy (petit mal)	1
Surditas	1
Headache	2
Enuresis nocturna	1
Walking troubles	2
Total	59

end of the period of investigation as at the beginning (Fig. 3). The remaining four of the fatal cases included one patient with purulent meningitis (meningococcica), one with thrombocytopenia (before contracting measles this patient had megaloblastic anemia and cerebral palsy), one with appendicitis and one with primary tuberculosis (besides measles this patient had whooping cough).

DISCUSSION

The period 1948–1962 was selected because it could be expected that the results would show that the prognosis had changed very much during this period. In 1952 an unusually severe poliomyelitis epidemic raged in Denmark during which the principles of treatment of respiratory failure were fundamentally changed. These new principles were naturally also applied if necessary to the treatment of measles. The appearance of the many new antibiotics would probably have changed the prognosis in bacterial complications especially in pneumonia.

The pneumonia mortality rate however is nearly the same throughout the whole period but may possibly decrease in the future as it is now feasible to make the bacteriological diagnosis so quickly that adequate therapy can be started within the first hour after admission to hospital. The bacteriological diagnosis is made by direct microscopy of stained smears from the trachea obtained by suction through a nasal catheter.

On the other hand the encephalitis mortality rate decreased remarkably during the period of

investigation. In the first three years five of 21 patients (24%) died from this complication while in the last 12 years of the period only one patient (2.1%) among 47 died. This decreasing mortality rate is no doubt due to the improved treatment in accordance with the experience from the polio epidemic. The innominate artery hemorrhage causing the only fatal case since 1951 is a known complication which has been seen twice among 623 cases of tracheostomy in Blegdams hospitalet (6).

The encephalitis mortality rate in the period of investigation was 9% which is on a par with other investigations (2). In a Swedish publication (1) one death occurred among 64 cases of encephalitis in the period 1955–1964. This period may be compared with the period 1953–1962 in the present investigation in which one death was found among 38 patients. The mortality rate is thus comparable with that of the Swedish publication.

The frequency of encephalitis was 0.43% of the notified cases in the City and County of Copenhagen. The incidence of encephalitis as a complication in measles is reported to range from 1% (1, 3) to 2.5% (5). The variations may be caused by varying criteria for the diagnosis. Some authors classify every symptom from the central nervous system as due to encephalitis which means that cases with secondary lymphocytic meningitis without other signs of central nervous disturbances than pleocytosis are included.

Encephalitis is a rare complication in measles and concerning mortality rate and sequelae is not so severe as considered previously.

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DISINFECTION OF THE HANDS OF WARD PERSONNEL

A Comparison of Six Disinfectants

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Abstract Six preparations for hand disinfection have been compared five hexachlorophene detergents and one quaternary ammonium compound. In preliminary experiments one of the hexachlorophene preparations and the quaternary ammonium compound were excluded from further investigations because of heavy skin irritating properties and poor antibacterial effect. The remaining four hexachlorophene preparations were compared using a Latin square design. Three of the preparations gave a 93.5-97.5 per cent reduction of the resident hand flora the fourth disinfectant being significantly less effective. One of the three preparations caused greater reduction in bacterial hand flora than the other disinfectants, which on the other hand have less side-effects. Altogether the differences between the three superior preparations were small and the cost may therefore partly decide which is to be chosen. During treatment with hexachlorophene preparations the frequency of Staphylococcus isolations was greatly reduced but there seemed to be no influence on gram negative organisms.

Disinfection of the hands is supposed to be one of the most important ways of controlling hospital infections (7, 14, 22). Various antiseptics have been found to reduce the bacterial flora of the hands markedly without causing serious skin irritation. These should therefore replace the use of ordinary soap by hospital staff and probably also by patients (2, 18). For such use hexachlorophene preparations seem to be the most suitable disinfectants at present.

However within this group of antiseptics there are great variations in quality and cost. It is well documented that the antibacterial effect of liquid preparations is superior to that of solid (8, 10) but the effect of liquid preparations also seems to vary (10). Some of these disinfectants are said to inhibit not only the growth of gram positive organisms but also to some extent the growth of gram negative rods. However the latter remains

to be proved in practical tests. Differences in side effects probably also exist (7).

Whilst dealing with prophylactic measures against surgical wound sepsis we have compared six hand washing preparations, five containing hexachlorophene and one based on a quaternary ammonium compound.

MATERIAL AND METHODS

The preparations numbered 1-6 (see below) were tested on 12 nurses and four laboratory technicians who during the treatment period used the preparations for all hand washing instead of soap. The experimental design is described in relation to each experiment. The bacteriological methods however were the same in all experiments.

The following antiseptic hand washing preparations were studied:

- 1 Zalpon (Izal) a liquid soap containing 2.5% hexachlorophene
- 2 Nicasept (AB Tyketa) a detergent containing 2% hexachlorophene
- 3 Sumasept (A/S Denofa og Lilleborg Fabrikker) a detergent containing 3% hexachlorophene
- 4 Phisoex (Winthrop) a detergent containing 3% hexachlorophene
- 5 Steraskin (Pfizer) a detergent containing 3% hexachlorophene and 3% chloroxylenol
- 6 Rodamun (Ferrosan) a detergent containing 1.2 benzalkonium chloride

The bacterial flora of the hands was investigated by a method described by Lowbury and Lilly (8) except for minor modifications. The subjects washed their hands in 300 ml of sampling fluid in the following way: both hands were moistened up to the wrist and then rubbed three times firmly to and fro first palm to palm then the right palm over the left dorsum the left palm over the right dorsum and last with the fingers interlacing followed by a final rinse. This was repeated three times and the whole procedure lasted two min.

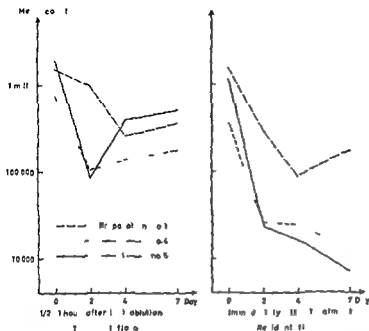


Fig 1 Mean counts of viable organisms before and during treatment with hexa chlorophene detergents

The transient flora was investigated by a method described by Story (19). A truncated glass cylinder was placed on the sampling area. Five ml of sampling fluid were measured into the cylinder and the skin was then systematically rubbed with a glass spreader for 30 sec.

Dilutions of sampling fluid were inoculated on lactose agar blood agar and mannitol salt agar plates which were incubated at 37 °C for 24, 40 and 48 h respectively. The selection of pathogenic staphylococci was based on the capacity to coagulate human plasma.

The sampling fluid was Ringer solution with 1 Tween 80. In inoculation tests this solution was found to neutralize the antibacterial effect of preparations 1, 2, 3 and 4. For preparations 5 and 6 tests for transfer of inhibitory concentrations of antiseptics to sampling fluid were made by inoculating control tubes and sampling fluid with dilutions of broth cultures of *Staph aureus*, *Staph albus* and *E. coli*. Counts made from pour plates did not indicate transfer of any inhibitory amounts of antiseptics to the sampling fluid.

EXPERIMENT I

Methods

Preparations 1, 4 and 5 were tested on five subjects who used the disinfectants for all hand washing for one week each, allowing one week of ordinary soap between each preparation for return of resident hand flora. The preparations were used in random sequence. Sampling by hand washing was done 1-1 hour after the last ablu- tion the subjects commu- directly from duty. Then the hands were washed with the preparation in a standard way under a running tap for one minute including a thorough rinsing and dried on a paper towel. A second sample of hand washing was then obtained.

Preparations 2, 4 and 6 were tested on four subjects in similar experiments except that the first sampling was omitted and that ordinary soap was used for the ablu- tion preceding the first day sampling.

Results

The left side of Fig. 1 shows the bacterial counts in hand washings from the subjects coming directly from duty representing mainly the transient flora (15). The right side of Fig. 1 gives the mean counts in hand washings obtained immediately after the treatment representing the resident flora (9-15). Both the transient and the resident flora were reduced during treatment. The individual counts from the transient flora show great fluctuations, most likely because of differences in external contamination between subjects and in the same individual at different times. Thus by these experiments it seems difficult to distinguish the effect of the different preparations and these samplings were therefore omitted in further investigations.

The results of the second sampling (resident flora) showed less fluctuations and suggested that preparation nos. 4 and 5 were superior to no. 1.

Table I shows the results of the second part of the experiment. The reduction in the counts of viable organisms with preparation 6 was very small. During treatment with no. 2, two out of four subjects experienced considerable skin ir-

Table I Mean counts (in thousands) of viable organisms in hand washings before and during treatment with three antiseptic detergents

Mean counts from four subjects

Preparation no	Before treatment	Day of treatment		
		2	4	7
2	758.4	140.2	102.0	174.8
4	778.9	35.2	15.2	25.1
6	515.9	106.8	328.7	350.0

irritation and as this detergent also caused only small bacterial reductions preparations 2 and 6 were excluded from further investigations

EXPERIMENT II

Transient Flora

Method

The residues of antiseptic on the skin after treatment with a preparation may kill some of the bacteria contaminating the skin. This was investigated by a method similar to that described by Lowbury *et al.* (11). A Latin square design was used to compare preparations 1, 3, 4 and 5 on four subjects each preparation being tested once on each individual. The subjects first rinsed their hands for one minute in 70% ethyl alcohol to reduce the resident flora. This was followed by two minutes treatment with the preparation under a running tap in a standard way including a final thorough rinse. The hands were dried on paper towels and immediately after wards, 0.02 ml of a 0-hour broth culture of *Staph aureus* was inoculated on a marked area of the palm of the left hand and spread over the area with a glass rod. The film of *Staph aureus* was allowed to dry and left untouched for one hour before the area was sampled by the method described above. The survival after washing with ordinary soap was elucidated in a separate experiment. The numbers of infecting organisms were calculated from dilutions of the cultures used.

As the frequency distribution of bacterial skin counts approximates the log normal form the counts were transformed to a logarithmic scale.

Results

Table II gives the counts of *Staph aureus* recovered. The logarithms of the number of infecting organisms varied from 6.76 to 6.86. The counts of *Staph aureus* recovered by the sampling expressed as a percentage of the number inoculated are given at the bottom of the table.

By analysis of variance the counts after treatment with the disinfectants were found not to differ significantly. However this limit of experi-

Table II Effect of residues of antiseptics on artificial contamination with *Staph aureus*

Counts (log) of recovered organisms

Subject no	Preparation no				Ordinary soap
	1	3	4	5	
1	5.04	5.50	4.57	4.70	6.39
2	4.63	4.49	3.09	4.06	6.31
3	5.59	4.93	5.12	4.61	6.6
4	5.15	3.25	3.31	3.42	6.70
Mean log count	5.10	4.54	4.0	4.20	6.43
Mean reduction (per cent of log count)	25	33	41	38	6

ment suggests that preparation no. 1 is less effective than the others. The superiority of disinfectants over ordinary soap is evident.

EXPERIMENT III

Resident Flora

Method

In this experiment preparations 1, 3, 4 and 5 were compared on 12 subjects. Each preparation was used for one week on each subject, allowing one week of ordinary soap between the treatment periods for return of the resident flora. A Latin square design was used in order to neutralize irrelevant factors. The subjects were divided into three groups, each group being allotted to different Latin squares. With only one week allowed for the return of the resident flora, the squares were arranged to neutralize 'carry-over' effect (3).

The resident flora was sampled after the standard hand washing with the detergent concerned. The first sample for each preparation being obtained immediately before the subjects started the treatment. Further samples were taken after 1, 4 and 7 days of use and 1 and 4 days after completing the treatment. As in the former experiments, the subjects were examined coming directly from duty 1 hour after the last ablution. The counts were transformed to a logarithmic scale.

Side-effects were recorded after interviewing and examinations at the end of the treatment with each preparation. The side-effects were given scores according to the scale at the bottom of table VI.

Results

Table III gives the counts (logarithms) of viable organisms in hand washings before treatment and during treatment. Subject no. 2 had to interrupt the treatment with preparation no. 1 because of heavy skin irritation after one day of use. In the statistical analysis the missing value has been estimated as stated by Cox (3).

Table III *Logarithms of counts of viable organisms in hand washings before and during treatment with hexachlorophene detergents*

Subject no	Preparation no							
	1		3		4		5	
	B	D	B	D	B	D	B	D
1	6.03	5.18	5.54	4.53	5.93	4.26	5.34	3.84
2	5.73	4.19 ^a	5.63	4.08	6.21	3.72	4.63	3.11
3	6.45	4.81	6.84	4.97	5.75	5.29	6.24	4.41
4	6.08	4.70	5.84	3.40	5.21	4.37	5.57	3.95
5	4.91	4.99	5.22	4.95	6.01	4.79	5.36	4.11
6	5.41	4.66	5.92	4.74	5.30	3.79	4.65	3.94
7	6.24	4.82	5.45	5.23	5.80	4.55	5.52	4.11
8	5.64	5.50	5.79	5.57	5.92	4.99	5.74	5.10
9	6.31	5.21	6.02	4.82	6.3	4.94	5.79	3.38
10	4.73	3.53	4.82	3.48	4.08	2.91	4.74	2.92
11	5.42	4.81	5.38	3.87	5.96	4.22	6.08	4.10
12	5.52	5.05	5.75	4.6	5.86	4.16	6.37	3.64
Mean log count	5.71	4.79	5.68	4.49	5.77	4.33	5.50	3.89
Mean count (not log)	899 200	175 700	1 009 800	92 800	776 200	65 400	641 000	27 700
Mean reduction	0.92		1.19		1.44		1.61	

B = before treatment D = during treatment

^a Estimated value (see text)

Fig 2 shows the mean counts from all subjects before during and after treatment with the different preparations. The effect of the preparations is compared by analysing the difference between the pretreatment counts and the mean counts during treatment (Table III). Using the studentized range test the allowance for the mean log counts is ± 0.621 ($P=0.05$). Thus the difference between preparations 1 and 5 is significant.

By analysis of variance the mean counts before treatment are not significantly different. Accordingly the mean counts during treatment may be compared directly. By the studentized range test the allowance is ± 0.408 ($P=0.05$). Accordingly preparation 5 is significantly better than all the others and preparation 4 is significantly superior to preparation 1. The differences between preparations 3 and 4 and between 1 and 3 are not significant.

The sum of the counts of *Staph aureus* in the hand washings from all subjects are shown in Table IV. None of the subjects were permanent carriers and five never yielded *Staph aureus* in their hand washings during the experiment. The results confirm that hexachlorophene treatment not only reduces the total bacterial counts in hand

washings markedly but also reduces the counts of the more transient *Staph aureus*.

Table V gives the sum of the counts of Gram negative bacilli in the hand washings. One nurse was permanently carrying Gram negative organisms throughout the experiments. The other isolations are most often from two or three consecutive samples from the same subjects, all but three subjects giving samples with Gram negative bacilli on one or another occasion. Evidently none of the preparations have any important effect on Gram negative bacilli on the hands.

Table VI shows the side effects experienced. There were usually slight with preparations 3, 4 and 5. By statistical analysis of the scores the allowance is ± 0.916 ($P=0.05$). Thus the side effects of preparations 3 and 4 are significantly less than the side effects of preparation 1. Preparation 3 also has significantly less side effects than preparation 5. The differences between preparations 3 and 4, between 4 and 5 and between 1 and 5 are not significant.

DISCUSSION

Antiseptic preparations for hand washing have been studied by many authors (e.g. 4, 8, 10, 11).

12 13 16) Several methods have been employed but as the conditions of the experiments often differ considerably from the practical use by hospital ward personnel (10) the results are not applicable to hospital ward routine. In this investigation however the experiments were especially designed to fit practical ward routine.

Hand-disinfecting agents should replace the use of ordinary soap by ward personnel and probably also by patients (2 18). The reduction of resident flora of the hands by regular use of hexachlorophene preparations is comparable with povidone iodine surgical scrub and alcoholic chlorhexidine (11 12). But as povidone iodine has all its effect at the time of application (12) and as alcoholic chlorhexidine or the chlorhexidine handlotion recommended by Rubbo and Gardner (17) is to be used after ordinary soap washing we find these preparations less suitable for ward personnel than the hexachlorophene preparations.

The effect of hexachlorophene in liquid detergents on resident flora of the hands is well documented. Our investigation documents significant differences between liquid preparations. During use of hexachlorophene containing compounds hexachlorophene is deposited in the skin (5 20) and the deposition in the skin depends on the vehicle of transmission (20); the differences between liquid preparations are easily explained.

Of the six antiseptics tested preparations 3 4 and 5 are thought to give satisfactory results with a reduction in the resident hand flora of 93.5-97.5.

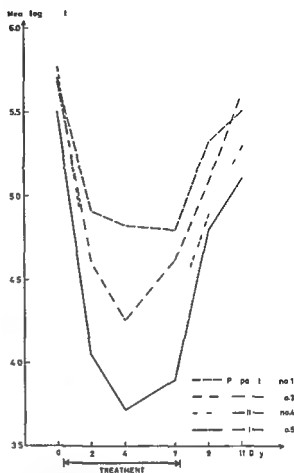


Fig. 2. Mean counts (logarithms) of viable organisms in hand washings before, during and after treatment with hexachlorophene detergents.

Table IV. Total counts (in thousands) of *Staph. aureus* in hand washings before and during treatment with hexachlorophene detergents.

Preparation no	Day before treatment			Day of treatment		
	5	3	0	4	7	
1	3.5 (1)	119.0 (2)	151.5 (2)	3.0 (2)	18.0 (2)	0.0 (0)
3	6.5 (3)	11.5 (3)	319.0 (3)	2.0 (2)	0.0 (0)	14.5 (1)
4	34.0 (1)	37.0 (3)	34.0 (3)	0.0 (0)	0.0 (0)	0.0 (0)
5	6.0 (1)	17.5 (1)	306.0 (3)	0.0 (0)	0.0 (0)	0.5 (1)
Total	10.0 (7)	180.0 (9)	810.5 (11)	5.0 (4)	18.0 (2)	15.0 (1)

Figures in parentheses indicate the number of isolations of *Staph. aureus*.

Table V Total counts (in thousands) of Gram negative bacilli in hand washings before and during treatment with hexachlorophene detergents

Preparation no	Day before treatment			Day of treatment		
	5	3	0	2	4	7
1	37.0 (1)	199.0 (1)	54.5 (2)	101.0 (2)	131.5 (1)	6.0 (3)
3	219.5 (1)	223.0 (9)	60.5 (4)	95.5 (2)	167.5 (1)	98.0 (1)
4	55.0 (2)	107.0 (2)	45.5 (1)	34.0 (4)	39.5 (1)	1.65 (1)
5	2.0 (3)	4.0 (2)	264.5 (2)	13.5 (1)	6.5 (1)	253.0 (2)
Total	333.5 (7)	533.0 (10)	425.0 (11)	244.0 (9)	345.0 (4)	503.5 (7)

Figures in parentheses indicate the number of isolations of Gram negative bacilli

Hexachlorophene preparations should be used regularly in order to get maximum effect (8-16-21). This is possibly demonstrated by the tendency of higher counts in hand washings after seven days of use than in the previous counts. As the last sampling during treatment was taken on Monday and the majority of the subjects were off duty on Sundays this probably reflects the less frequent usage of the preparations off duty.

The preliminary experiments suggest that treatment with hexachlorophene preparations has a suppressive action on the transient flora of the

hands. This is confirmed by the experiment with inoculation of *Staph. aureus* on the hands after treatment. The effect of hexachlorophene preparations used only on duty has been doubted (13-21). However the inoculation experiment demonstrates a considerable effect of residues of antiseptic left on skin after a single thorough hand washing with the preparations. Thus it seems desirable that all personnel are instructed to wash their hands thoroughly at the beginning of the day.

Gram negative bacilli represent an increasing danger in hospitals. Hexachlorophene is less effective against gram negative bacilli and may even sometimes promote colonization of the skin by gram negative organisms (17). In vitro tests of chloroxylonol (1-17) and preparation 1 suggest that these have some effect also against Gram negative bacilli. The present investigation however fails to demonstrate any effect on this flora.

Detergents with hexachlorophene are often reported to cause some dryness of the skin but allergic reactions seem rare (8-10). The differences in side effects between the preparations demonstrated by this investigation are important when disinfectants for long term use are to be chosen.

Table VI Side effects (skin irritation) of hexachlorophene detergents

Subject no	Preparation no			
	1	3	4	5
1	0	1	1	0
2	4	0	0	2
3	1	0	0	0
4	2	1	1	1
5	3	1	2	2
6	1	2	1	1
7	2	1	1	1
8	3	0	1	2
9	3	0	1	2
10	2	0	1	3
11	2	0	1	2
12	2	1	1	2
Mean score	2.08	0.58	0.97	1.50

0 = no side-effects

1 = dryness of skin

2 = 1 plus slight irritation (redness or soreness)

3 = considerable irritation (redness and soreness)

4 = heavy irritation causing interruption of treatment

ACKNOWLEDGEMENTS

The financial support which enabled the investigation to be carried out was provided by Sosialdepartementet, HelseDirektoratet, and by a grant from Norsk Medicinaldepot.

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ATRIAL FIBRILLATION

A Review of 463 Cases from Philadelphia General Hospital from 1955 to 1965

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Abstract The hospital records of 463 patients with atrial fibrillation occurring over a 10-year period at the Philadelphia General Hospital have been reviewed. The different etiologic factors have been determined as well as some sexual and racial proportions. Criteria of the different groupings are explained and some associated ECG findings reported.

Arteriosclerotic and hypertensive heart diseases are responsible for atrial fibrillation in this study in almost 65% of the cases.

The incidence of embolic episodes in the different etiologic groups is also compared. For the entire series the percentage was 17.9.

The purpose of this report is to review some of the pertinent clinical features of atrial fibrillation. Particular attention is paid to the probable etiologic background or associated disease concomitant electrocardiographic findings and finally embolic complications. Very few authors have reported recently on these aspects of atrial fibrillation although therapy particularly counter shock has been extensively discussed.

Since atrial fibrillation is a common phenomenon and many of its clinical features change simultaneously with changes in the epidemiology of this arrhythmia a review of these changes is considered justifiable. Such a recent epidemiologic study is necessary to evaluate the representativeness of the materials now published in which countershock has been used to convert atrial fibrillation.

MATERIAL AND METHODS

The study was based on a review of the hospital records with a diagnosis of atrial fibrillation during a 10-

year period (January 1 1955 to December 31 1964) at the Philadelphia General Hospital. No cases were included which did not have an ECG tracing available at the time of review which accurately established the diagnosis of atrial fibrillation.

The following data were determined in each case: age sex race associated and/or etiologic diseases some concomitant ECG findings and finally frequency of embolism.

Age

The age of the patient was recorded at time of onset of atrial fibrillation regardless of whether this occurred before 1955 or during the 10-year period of study.

Etiologic factors

Since atrial fibrillation with few exceptions occurs primarily in elderly patients there was reason to believe that frequently more than one etiologic factor exists. Consequently many reports are affected by this drawback. However by presenting combination groups of arteriosclerosis and hypertension rheumatic heart disease and arteriosclerosis a more accurate grouping was obtained. The criteria of the different groupings in the present study are given below.

1 Arteriosclerotic heart disease. There was frequently a positive family history of this disease or evidence of angina pectoris myocardial infarction or diabetes. These patients often presented congestive heart failure.

2 Hypertensive cardiovascular disease. Hypertension was diagnosed when the blood pressure readings were at least 100 mm Hg diastolically (1-3). One exception however was a diabetic patient with elevated blood pressure. This patient was therefore included in group 1 (Fig. 1-6).

3 Rheumatic etiology was considered in a subject with a history of rheumatic fever or a rheumatic type of infection. Valvular lesions mitral and aortic were present either alone or combined.

4 Arteriosclerosis and hypertension diagnosed as above occurring in the same patient.

5 Similarly rheumatic heart disease and arteriosclerosis combined in the same patient.

6 Thyrotoxicosis required a clinical picture of the disease positive tests for thyroid overactivity such as

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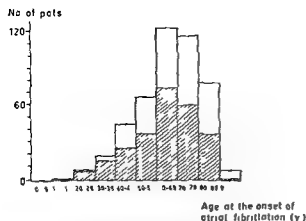


Fig 1 Number of patients in different age groups at time of onset of atrial fibrillation. Blank columns denote number of females and hatched columns denote males

elevated protein bound iodine, increased thyroid uptake of I^{131} and an elevated basal metabolic rate

7 Atrial fibrillation associated with syphilitic aortitis. This diagnosis was made when there was a classical aortic insufficiency, a positive serology test for syphilis and in all cases a history of luetic infection. (In two cases the diagnosis was confirmed at autopsy)

8 Alcoholic myocardial disease. All these patients had a long history of excessive alcohol intake. Often and contrary to beriberi heart disease the food intake in this group of patients seemed to be fairly adequate. None of these patients responded to thiamine therapy (9, 7, 25)

9 Idiopathic atrial fibrillation was considered as the diagnosis in the complete absence of heart disease after extensive medical evaluation (13, 20)

10 Severe infections. This group was more heterogeneous in composition. In our cases the infection was the dominating feature in the patient's clinical picture: Gram negative septicemia, chronic osteomyelitis, lobar pneumonia and extensive tuberculosis involving the heart were the diagnoses in these cases

11 Miscellaneous. A few cases were observed with extensive pulmonary neoplasms with pathologic anatomic evidence of spread to the heart. There was also

Table I Age distribution of the entire material with atrial fibrillation

Age groups (y)	Males	Females	Total
10-19		2	2
20-29	7	1	8
30-39	15	3	18
40-49	24	19	43
50-59	36	32	68
60-69	73	48	121
70-79	60	57	117
80-89	36	42	78
90-99	2	6	8
Total no of pat	253	210	463

one case with atrial fibrillation that started immediately after lobectomy due to pulmonary tuberculosis. One young female developed atrial fibrillation during the last trimester of two successive pregnancies without clinical evidence of valvular disease or thyrotoxicosis (19). One case of a congenital heart lesion with a familial rhythm disturbance was discovered (15). One patient with a myocardiopathy probably secondary to lupus erythematosus was also placed in this group. A few cases were included with moderate to severe degree of pulmonary emphysema associated with atrial fibrillation.

Associated ECG findings

The following findings were recorded: myocardial infarctions, recent or old complete heart block, left and right bundle branch block, left and right ventricular hypertrophy. The hypertrophy was diagnosed according to the criteria applied by Sokolow and Lyon and in most cases was supported by vectorcardiography (29, 30)

Complications with thromboembolic phenomena

Only patients with a well-documented hospital record of an embolic episode were included. These emboli occurred either prior to or during the present admission. The embolization was divided into cerebral, pulmonary and peripheral (leg, arm, and abdomen) groups. How

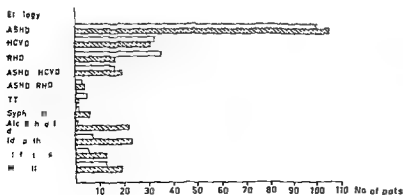


Fig 2 Number of patients in the different etiological groups. Blank columns denote number of females and hatched columns denote males

ever the difficulty of antemortem separation of cerebral embolism and thrombosis is well known and should be remembered.

Statistical method

In the statistical analysis the following probability levels of significance were used $p < 0.001$ was highly significant * $0.001 < p < 0.01$ significant ° and $0.01 < p < 0.05$ probably significant The means were given \pm the standard error of the mean (1°)

RESULTS

Age sex and race

The entire material consisted of 463 patients The mean age was 65.2 ± 1.0 years There were 253 males and 210 females the ratio of males to females was 1.2:1.0 The mean age of the males was 63.3 ± 1.0 years range 20–91 years and that of females 67.4 ± 1.0 years range 12–97 years (Fig 1 and Table I)

Etiologic factors

The clinical diagnoses obtained are shown in Fig 2 and Table II

Associated ECG findings

Myocardial infarctions were diagnosed in 9.5% of the entire series Of these 11.4% were acute Left bundle branch block was registered in 7.1% and complete heart block in 1.1% Left bundle branch block occurred in 10% of the cases with arteriosclerotic heart disease and in 6.3% of the cases with hypertensive cardiovascular disease Left ventricular hypertrophy was observed in 25.1% of the entire series and right ventricular hypertrophy in 2.4% In the group with rheumatic heart disease however right ventricular hypertrophy was seen in 10.7% and left ventricular hypertrophy in 39.3% (Table III)

Thromboembolic complications

The incidence of embolization is recorded in Table IV As a percentage of the entire series this was 17.9

DISCUSSION

Comparison with other studies

The size of this series is comparable to some of the larger series that have been previously reported (18, 22, 28, 31) The material consists of only patients admitted and consequently patients

Table II The different etiologic factors (total numbers, races, mean ages and percentages)

Etiology	Mean age												Percentage								
	♂	♀	♂	♀	♂ + ♀	♂	♀	♂ + ♀	♂	♀	♂ + ♀	♂ + ♀	♂ + ♀	♂ + ♀							
ASHD	71	32	63	34	134	66	103	97	200	73.0	69.8	76.2	71.6	74.5	70.8	7.0	74.6	73.3	33.3	50.7	43.3
HCVD	7	7	19	14	76	38	31	33	64	77.4	61.4	76.0	60.0	75.0	60.9	63.9	69.4	66.4	19.1	9.8	13.8
RHD	5	11	15	20	20	31	16	35	51	4.4	47.4	35.7	51.2	57.4	49.8	45.8	53.1	50.8	15.6	7.6	11.0
ASHD + HCVD	11	8	7	9	18	18	19	16	35	68.5	65.0	73.1	65.2	70.3	65.1	67.0	68.6	67.8	9.0	6.8	7.8
ASHD + RHD	1	2	2	3	3	2	3	5	29	38.5	38.5	59.0	—	49.0	38.5	35.3	59.0	44.8	1.0	1.1	1.1
TT	—	1	1	3	1	4	1	4	5	75.0	52.0	68.3	—	67.0	—	68.0	68.2	67.0	2.0	0.4	1.1
Syph aort	—	5	—	1	—	6	5	1	6	—	68.7	—	—	—	—	68.0	68.2	67.0	3.0	—	—
Alcoh heart dis	17	4	1	—	18	4	7	1	22	55.5	50.0	68.0	—	56.2	50.0	54.4	68.0	55.2	2.0	6.8	4.7
Idiopathic	14	8	—	6	14	14	22	6	78	45.6	37.0	—	43.7	45.6	39.9	42.5	43.7	42.7	7.0	5.3	6.0
Infectious	9	3	4	—	13	3	12	4	16	62.7	69.3	75.0	—	66.5	69.3	64.3	75.0	67.1	3.5	4.9	3.4
Miscellaneous	10	9	7	5	17	14	19	12	31	61.4	64.6	64.6	45.8	62.7	64.1	64.1	56.8	61.3	6.5	6.4	6.5
Total	144	107	110	92	764	199	51	212	463	64.2	61.2	71.5	61.7	67.6	61	63.3	67.4	65.2	100.0	100.0	100.0

Open circles in sex symbols denote Caucasians filled circles denote Negroes
 s.e. = standard error ASHD = arteriosclerotic heart disease HCVD = hypertensive cardiovascular disease RHD = rheumatic heart disease TT = thyrotoxicosis Syph aort = syphilitic aortitis Alcoh heart dis = alcoholic heart disease (myocardiodiopathy)

Table III Associated ECG findings in three etiologic groups

Etiology	No of pats	Myocardial infarction (old or new)	LBBB	RBBB	Per cent of recorded ECG findings in entire material
ASHD	200	19 (9.5 %)	20 (10.0 %)	1 (0.5 %)	25.5
HCVD	64	—	4 (6.3 %)	5 (7.8 %)	14.1
RHD	51	—	1 (2.0 %)	5 (9.8 %)	11.8

LBBB = left bundle branch block RBBB = right bundle branch block Others as in Table II

dying in the outpatient department and those visiting on an ambulatory basis were excluded

A General Hospital population represents in regard to the socio-economic status of the patients a certain type of selection. However this type of hospital population reflects a large proportion of the general population. Previous materials were also mainly selected from this type of hospital.

Age at onset of atrial fibrillation was similar in my group to that of the material reported on by Sawyer et al (28). The peak incidence in the present study was in the 60-70 age group. Earlier reports have shown a lower mean age. Most studies report a higher incidence of atrial fibrillation among males than among females. The male to female ratio in my study was 1.2:1.

Earlier studies have not reported any racial ratios and therefore a comparison is not possible.

Etiology

The difficulties at times of determining a single etiology of atrial fibrillation were evident during

this study. Particular attention was paid to differentiating between arteriosclerotic and hypertensive heart disease. This difficult differential diagnosis is apparent since hypertension has been reported to occur in as high a proportion as 50% in men and in about 75% in women with coronary heart disease (16, 17). On the other hand hypertensive patients have a high incidence of coronary heart disease. Some reports have therefore not separated these diseases but combined them as etiologic factors.

In comparison with previous reports there is an expected change in the more recent studies (Table V) (10, 18, 22, 28). The increased role of arteriosclerotic and hypertensive heart disease was observed. This trend is probably due to the increased number of patients with arteriosclerotic heart disease as the mean age of the population is increasing. Other important factors are the decrease of rheumatic fever and the diminished severity of the disease. These changes began in the late 1930s before the antibiotic era (4). As there is a long interval between the attack of

Table IV Embolic episodes in the different etiologic groups

Etiology	No of pats	Cerebral emboli	Pulmonary emboli	Peripheral emboli	No of pats with > 1 embolus	No of pats with emboli	Per cent of pats with emboli in entire material
ASHD	200	30	8	4	3	45	22.5
HCVD	64	19	1	—	—	22	34.4
RHD	51	4	4	2	—	11	21.6
ASHD + HCVD	35	5	1	—	—	6	17.1
ASHD + RHD	5	—	—	1	—	1	20.0
TT	5	1	—	—	—	1	20.0
Syst. aort.	6	—	—	—	—	—	—
Alcohol heart dis.	22	—	1	—	—	1	4.5
Idiopathic	28	—	—	—	—	—	—
Infectious	16	—	—	—	—	—	—
Miscellaneous	31	1	—	—	—	1	3
Total	463	61	17	6	3	80	17.9

Abbreviations as in Table II

Table V The etiology of atrial fibrillation in five series

Numbers are per cent of total

Authors	Åberg 1968	Sawyer 1956	Goldman 1951	McEachern and Baker 1932	Parkinson and Campbell 1930
No. of pat.	463	407	80	575	200
<i>Etiology</i>					
ASHD	43.3	36.1	55.0	31.1	2.5
HCVD	13.8	9.3		16.9	24.0
RHD	11.0	29.7	31.25	34.4	2.0
ASHD + HCVD	7.8	12.3	—	—	—
ASHD + RHD	1.1	1.5	—	—	—
TT	1.1	7.6	5.0	7.5	14.0
Syph. aort.	1.3	—	12.5	3.0	2.5
Miscellaneous	6.5	3.4	7.5	2.1	15.0
Other groups	14.1	0.1	—	5.0	—

Abbreviations as in Table II

rheumatic fever and established rheumatic valvular disease with atrial fibrillation, it is readily accepted that the decrease in rheumatic fever in the late 1930s and the early 1940s is responsible for the statistical change at the time of the present study. It also explains the higher mean age in the group of rheumatic heart disease in this series compared with earlier studies. The clinical view that atrial fibrillation in a young patient is most likely caused by rheumatic heart disease is no longer as valid as in previous generations.

The sex ratio is almost identical in both the arteriosclerotic group and the hypertensive group, namely 1:1 (Table II). In the arteriosclerotic group there are more males in the comparatively low age groups. In the group below 55 years of age there are nine males and three females.

As shown in Table II there is a striking difference between the races regarding the most common cause of atrial fibrillation. In the white population hypertensive heart disease is the cause in 98% of the cases but in the Negro it represents 19.1%. This difference is highly significant (). It is well known that hypertension is seen more frequently and in a more severe form among the Negroes in the United States than among the Caucasians (34). The reverse is true, however, in the group where arteriosclerosis is the cause. In the white group the figure is 50.7% and in the Negro 33.3%. This difference is also highly significant (). The different epidemiology among the races in the United States has

been reported earlier (24). The high incidence of hypertension without arteriosclerosis in this series is surprising. Of course there is the possibility of undetected arteriosclerotic heart disease in the hypertensive group. The high ratio of Negroes in the series is another explanation.

Hyperthyroidism occurred in 1.1% of the cases of atrial fibrillation. This percentage is lower than in earlier reports. As most cases with thyrotoxicosis occur during the third and fourth decades of life it is remarkable that the mean age of thyrotoxic heart disease manifested in atrial fibrillation is much higher. This observation has led some authors to believe that hyperthyroidism alone does not cause atrial fibrillation without the complication of some additional organic heart disease (27).

A few years ago Brigden and Robertson described the alcoholic myocardiopathy which seems to be a cardiac manifestation of alcoholism other than beriberi heart disease (5, 7, 25). These authors report rapid atrial fibrillation in half of the patients at some time during the illness. The cause might be changes in the distal branches of the coronary arteries, particularly those located in the atria (25). The size of this etiologic group will necessarily depend upon the composition of the hospital population. My impression is that this group is overrepresented in the series under review. No comparison of this group with other studies is possible as the disease has not been recognized as an etiologic factor in earlier reports.

Considerable emphasis has been given to the

rarity of atrial fibrillation in syphilitic cardiovascular disease. In our series 1.3% of the cases of atrial fibrillation were associated with syphilitic aortitis which is within the same range as in previous reports.

Idiopathic atrial fibrillation originally described by Gossage and Hicks in 1913 (11) is more common in males. I found a male to female ratio of 4:1. Phillips and Levine (23) and Orgain et al (21) have stated that idiopathic atrial fibrillation can be expected to occur in slightly more than 5% of all cases of atrial fibrillation. My result of 1.3% is in very close agreement with the above prediction.

The author observed only one case of atrial fibrillation that occurred during childhood. This was a case of transposition of the great vessels in a 12-year-old girl who presented familial congenital atrioventricular dissociation and atrial fibrillation (15). Cookson in his large series of atrial fibrillation consisting of 1164 cases had only 2.5% below the age of 17 and no instances below the age of 12 (6). In 500 cases Thurmann found only four with congenital heart disease as the cause of atrial fibrillation. Of these patients three had atrial septum defects either alone or combined with other defects (33).

Thromboembolic phenomena

Embolus formation in atrial fibrillation is often discussed and only in rheumatic mitral disease is there general agreement that fibrillation increases the risk. The question of anticoagulation therapy is accordingly controversial in atrial fibrillation due to other causes (2, 9).

The high incidence of emboli in the arteriosclerotic and hypertensive groups is noticeable. I would suspect that some of the arteriosclerotic hypertensive patients did in fact experience thrombotic rather than embolic obstructions.

In the rheumatic group the incidence of embolic phenomena is lower than reported for some series. This might be explained to some extent by the fact that the author was unable to make a follow-up study. The expected higher incidence of cerebral and peripheral emboli in comparison with pulmonary emboli in rheumatic heart disease and atrial fibrillation is not as impressive as in the report by Sawyer et al (28).

In the arteriosclerotic and hypertensive groups

there is a surprisingly high occurrence of cerebral and peripheral emboli. This is in contrast to reports from necropsy studies in which it has been shown that in arteriosclerotic heart disease there are more thrombi present in the right atrium than in the left (14, 32). The possible explanation for the high ratio of cerebral and peripheral emboli to pulmonary emboli is that pulmonary emboli occur more frequently without being diagnosed.

ACKNOWLEDGEMENT

This work was aided by grants from the National Institutes of Health, Bethesda Maryland USA.

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DIRECT CURRENT CONVERSION OF ATRIAL FIBRILLATION— LONG TERM RESULTS

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Abstract During a 4-year period (August 1963 to September 1967) 181 patients with atrial fibrillation were treated with direct current countershock technique.

The patients have been followed during 5 to 5 months. The immediate and the long-term results are reported. The long-term results are disappointing: only 2 % of those reverted remain in sinus rhythm after one year. Factors which might influence the result are studied. Finally indications and contraindications for conversion of atrial fibrillation are discussed.

Direct current countershock has become a valuable method in the treatment of some arrhythmias. Since Lown et al introduced the method (19) it has gained widespread use. Conversion of atrial fibrillation to sinus rhythm is desirable for many reasons. The hemodynamic effectiveness of the heart is generally improved, especially during exercise (10, 26, 33, 35, 37). Further, it is generally accepted that restored sinus rhythm decreases the considerable risk of systemic and pulmonary embolism, particularly in rheumatic heart disease (39).

Synchronized DC shock is a safe and effective method to restore atrial fibrillation to sinus rhythm (1, 4, 7-9, 11, 13, 16-20, 23-25, 27-29, 34). Thus the decisive factors whether or not an attempt should be made to convert atrial fibrillation will mainly be the estimated risks and the possibility of maintaining sinus rhythm. As with all new therapeutic methods thorough follow-up studies are necessary to define the indications.

The aim of this paper is to report on our long-term results after DC conversion of atrial fibrillation.

MATERIAL AND METHODS

From August 1963 to August 1967 181 patients with atrial fibrillation have been treated with DC-shock in

our department. The pertinent clinical data on the material are shown in Table I.

The patients with arteriosclerotic heart disease had a history of angina pectoris, earlier myocardial infarction, hypertension or diabetes. Only one of these patients was under 50 years of age.

The diagnosis of rheumatic valvular disease was established by cardiac catheterization, angiocardigraphy and/or surgery in 81.5 % and based on clinical findings in 18.5 %. Patients with severe mitral stenosis and other valvular diseases were not converted prior to surgery. Of the 68 patients with mitral commissurotomy 57 % were converted 4-8 weeks after surgery. A ball valve prosthesis was inserted in 11 cases.

In the miscellaneous group two patients had ventricular septal defects, one patent ductus arteriosus, one coronary artery aneurysm, and one hypertrophic subvalvular aortic stenosis. In one patient the ventricular septal defect was combined with pulmonary stenosis. All these patients underwent corrective surgery prior to conversion.

The diagnosis of myocarditis was based on the arrhythmia, the sudden development of cardiac enlargement and heart failure in the absence of valvular and arteriosclerotic heart disease.

Four patients had persistent atrial fibrillation after successful treatment of hyperthyroidism. Patients with hyperthyroidism were not treated for atrial fibrillation until they had become euthyroid.

The remaining group was diagnosed as "idiopathic" when there was no evidence of heart disease.

The duration of atrial fibrillation, prior to the DC shock, has been determined as the time during which the patient definitely noticed an irregular heart activity. If the patient was unable to give a date we have recorded the first time the arrhythmia was diagnosed by a physician.

Many patients have been converted several times. The result of the patient's first conversion or where two or more attempts were made during the same admission, the last one has been tabularized.

The technique used for this material, has been previously reported (1, 7). All conversions were performed on hospitalized patients. The patient was in a supine position. A short acting barbiturate (Pentothal®) intravenously administered was used as an anesthetic. Digitalis was discontinued 48-72 hours before the DC-shock.

Table I Total group of patients with atrial fibrillation where conversion was attempted

Etiology	Number of cases			Age (y)		Heart volume (ml/m ² BSA)	
	♂	♀	Total	Mean	Range	Mean	Range
ASHD	28	17	45	62	48-73	601	410-790
MS							
Operated	11	28	39	49	32-63	633 ^a	470-810
Not operated	3	3	6	57	43-68	616	415-750
MI							
Operated	1	3	4	44	37-53	665	550-850
Not operated	3	1	4	50	35-64	735	600-940
MS + MI							
Operated	8	8	16	46	36-55	672	445-930
Not operated	6	9	15	52	44-62	675	480-1110
AS - AI							
Operated	2	1	3	50	42-58	747	640-900
Not operated	1	—	1	—	60	—	680
M + A							
Operated	8	14	22	46	37-56	714	480-1000
Not operated	3	3	6	51	29-63	765	560-1020
Miscellaneous	2	6	8	48	34-60	736	590-870
Myocarditis	5	—	5	48	41-54	654	50-760
Treated hyperthyroidism	—	4	4	64	58-71	560	470-630
Idiopathic	3	—	3	41	40-44	483	440-510
Total	84	97	181	54	29-73	693	410-1110

^a One patient's heart volume not known

Abbreviations: ASHD = arteriosclerotic heart disease; MS = mitral stenosis; MI = mitral insufficiency; AS - AI = aortic stenosis and/or aortic insufficiency; M + A = mitral and aortic lesions

Until late 1966 quinidine was given a few days prior to conversion. In most cases we used the Cardiac Synchronizer (Corbin Farnsworth Company). One shock was given at each of the energy levels 80, 100, 200, 300 and up to 3 attempts at 400 Ws. This procedure was not strictly followed in all cases.

With regard to maintenance therapy quinidine was given as a rule until the beginning of 1967 when other investigations were started in an attempt to develop a better prophylactic therapy. These will be published elsewhere (?) The quinidine dose was adjusted according to the serum level of quinidine (12) aiming at a concentration of 5-7 mg/l.

The roentgenological heart volume was measured by the method described by Jonell (14) during the same admission when the conversion was performed.

RESULTS

Immediate results

In 181 patients 290 attempts at conversion were made. Sinus rhythm for a period of at least 30 seconds was obtained in 146 patients. The convertibility rate was 81.2%.

There were more failures among patients with atrial fibrillation of long duration. On the other hand there was no difference between this group

of failures and those converted in regard to heart volume or age (Tables III and IV). In the valvular disease group there are only four failures out of 20 with a heart volume exceeding 750 ml/m² BSA. These four patients had atrial fibrillation of long duration: 13, 10, 3 and 1½ years respectively.

The total number of shocks administered was 486. In the group with successful conversions the number of shocks on each occasion was not significantly related to sex, age, etiology or maintenance of sinus rhythm after the conversion. When comparing different body weights a trend is apparent: patients weighing more required more shocks. The difference however is not significant.

Long term results

The follow up period varied from 5 months to 52 months. Three patients out of 181 did not take part in the follow up. Two of these could not be reached. One of them was a Polish citizen. The third patient had sinus rhythm 6½ months after conversion but was later killed in a traffic accident.

Table II Persistence of sinus rhythm after conversion in relation to etiology

Etiology	Total	Failures	Period with sinus rhythm				
			< 24 h	24 h - 1 mo	1-3 mo	3-12 mo	> 1 y
No. of patients							
ASHD	44	11	7	7	6	6	7
MS							
Operated	39	4	5	14	3	10	3
Not operated	8	—	—	—	—	3	3
MI							
Operated	4	2	—	—	1	—	1
Not operated	4	—	1	—	—	1	2
MS + MI							
Operated	16	3	2	6	1	—	4
Not operated	15	6	1	6	—	1	1
A - M + A							
Operated	24	5	2	9	2	3	3
Not operated	7	1	—	—	2	3	1
Miscellaneous	7 ^a	—	—	2	1	—	4
Myocarditis	5	1	—	1	—	2	1
Treated hyperthyroidism	4	2	1	—	—	—	1
Idiopathic	3	—	—	2	—	—	1
Total	178	35	19	47	16	29	32

One patient lost to follow up

Abbreviations A - M + A = aortic or aortic and mitral valvular disease Others as in table I

Twenty-eight of 178 patients still remain in sinus rhythm with an average follow up period of 1 year 6 months and 10 days range 5 months to 36 months Table II shows the persistence of sinus rhythm after conversion in the different etiologic groups in Table III it is related to the duration of atrial fibrillation and in Table IV to

the heart volume It is apparent that the duration of the fibrillation is of utmost importance in practically all etiologic groups On the other hand the heart volume seems to be less important as a cause of relapse The high frequency of relapse in operated mitral stenosis is evident.

More than one attempt at conversion was

Table III Persistence of sinus rhythm after conversion correlated with diagnosis and duration of atrial fibrillation

Etiology	Total	Period with sinus rhythm														
		Failures			< 1 mo			1-3 mo			3-12 mo			> 1 y		
		S	M	L	S	M	L	S	M	L	S	M	L	S	M	L
ASH	41 ^a	1	4	6	3	5	4	4	1	1	1	1	3	3	—	2
MS	41 ^a	—	—	4	3	2	13	1	1	—	1	4	7	—	—	3
MI	8	—	—	2	—	—	1	—	—	1	—	—	1	3	—	—
MS + MI	30 ¹	1	1	7	5	2	8	—	—	1	—	1	—	1	—	3
A - M + A	31	—	1	5	2	4	5	—	—	1	6	—	—	1	—	1
Miscellaneous	6	—	—	—	1	—	—	—	1	—	—	—	—	—	—	2
Myocarditis	5	1	—	—	1	—	—	—	—	—	1	—	1	—	1	—
Treated hyperthyroidism	4	—	1	1	1	—	—	—	—	—	—	—	—	—	1	—
Idiopathic	3	—	—	—	1	1	—	—	—	—	—	—	—	—	1	—
Total	169	3	7	5	17	18	31	7	3	5	9	6	12	8	11	11

An asterisk preceding a number denotes the number of patients with unknown duration of atrial fibrillation

Abbreviations S - short = < 6 months. M - medium = 6-24 months. L - long = > 2 years. Others as in Tables I and II

Table IV Persistence of sinus rhythm after conversion correlated with diagnosis and heart volume

Etiology	Total	Period with sinus rhythm														
		Failures			< 1 mo			1-3 mo			3-12 mo			> 1 y		
		H	M	L	S	M	L	S	M	L	S	M	L	S	M	L
ASHD	44	1	8	2	3	III	2	1	4	—	2	4	—	3	4	—
MS	44 ¹	—	4	—	2	12	4	1	2	—	5	7	1	4	1	1
MI	8	—	2	—	—	1	—	—	1	—	—	—	1	—	1	1
MS + MI	31	1	5	3	2	10	3	—	—	1	1	—	—	—	2	1
A - M + A	30 ²	—	3	2	1	6	3	—	—	3	—	3	2	—	5	—
Miscellaneous	7	—	—	—	—	1	1	—	1	—	—	—	—	—	2	2
Myocarditis	5	—	1	—	—	1	—	—	—	—	—	1	1	1	—	—
Treated hyperthyroidism	4	1	1	—	—	1	—	—	—	—	—	—	—	1	—	—
Idiopathic	3	—	—	—	2	—	—	—	—	—	—	—	—	1	—	—
Total	176 ³	3	24	7	10	42	13	2	10	4	8	15	5	14	15	6

An asterisk preceding a number denotes the number of patients who did not have a chest X ray on the same admission as the conversion

Abbreviations: S = small = < 550 ml/m² BSA M = medium = 550-750 ml/m² BSA L = large = > 750 ml/m² BSA Others as in Tables I and II

made in 68 cases of which 66 could be followed. In seven cases sinus rhythm was never obtained. In 36 of the remaining 59 cases no attempt resulted in sinus rhythm lasting longer than 3 months. Only 12 cases were in sinus rhythm after the last attempt with an observation period of 2 months to 2 1/2 years and where 33 conversions had been performed. More than three conversions had been performed in nine cases. It is of interest that only in six cases was there a

major difference in the outcome of the conversions as regards persistence of sinus rhythm.

One hundred and six patients received quinidine as maintenance therapy. In three cases the maintenance dose was not adequate and six patients discontinued the drug either of their own accord or on a physician's recommendation. Of the 97 patients who received adequate quinidine treatment only 27% remained in sinus rhythm after 3 months.

Table V Long term results in different reports

Authors	No of patients converted to sinus rhythm	Convertibility rate (%)	Proportion valvular disease to others	Percentage remaining in sinus rhythm at different times						
				1 mo	2 mo	3 mo	4 mo	1 y	2 y	
Rabbino et al (31)	31	88	33	1		32				
Korsgren et al (18)	107	78	14	1			40			
Killip and Yormak (17)	115	87			60			27		
Halmos (8)	137	78	40	1				(4)		
Hurst and Logue (13)	121	96 ^b	05	1		55				
Morris et al (27)	100	93	19	1		87				
Eberdt et al (8)	35	III	16	1	57	46				
Futrel and McGuire (9)	42	III	21	1		45				
Wikland et al (40)	60 ^d	81	06	1	60			25		
Radford and Evans (32)	119	77	12	1		47		18	13	
Present study	146	81	2	1	55	4		22	11	

Follow up period 9 months instead of 1 year

^b Convertibility rate measured for many attempts

^c This number stands for 117 patients who tolerated maintenance quinidine therapy. Furthermore some patients reverted without shock.

^d Eight patients had atrial flutter

The patients who did not receive quinidine were discharged without therapy in two cases and were given procainamide in 13 cases. In this small combined group 47% remained in sinus rhythm after 3 months. We have also studied the whole material with regard to earlier conversions with quinidine. Sixty three patients out of 181 had previously been reverted by quinidine. There were 12 cases where DC shock failed but quinidine had earlier proved successful. However it is not possible to draw any conclusions since there was a time lapse between the different attempts with the possibility of deterioration of the heart disease.

Complications

These will not be discussed as they have recently been published by our department (1). Only one point should be stressed. During the first 3 years we had eight cases with ventricular tachycardia or ventricular fibrillation in immediate relation to the shock. Since one and a half year ago when we abandoned administration of quinidine prior to the DC shock we have not had a single case with this severe complication.

DISCUSSION

Our case material is not representative of an unselected sample of atrial fibrillation (3). There is a predominance of patients with operated heart disease of both rheumatic and congenital etiology. This is due to the active thoracic surgery performed in Uppsala during the time of this study. In Table V the composition of the diagnoses is compared with that of other DC series.

The mean duration of atrial fibrillation in our series 2 years and 10 months is in good agreement with that of other authors (18, 22, 28, 34, 40). It is important to recognize this as on the whole there seems to be a correlation between the duration of fibrillation and the long term result.

The percentage of successful conversions 81.2% is approximately the same or slightly lower than that in other series (8, 9, 22, 27, 28). The differences mainly reflect the various criteria used for successful conversion. In some series the convertibility rate is lower but there as a rule conversion was only regarded as successful when

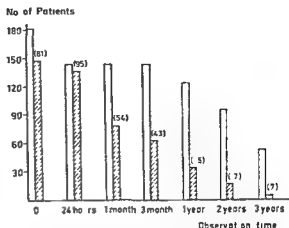


Fig 1 Number of patients in sinus rhythm after different follow up times correlated to total number at risk. Blank column denotes total number of patients at risk at different observation periods. Hatched column denotes total number still in sinus rhythm. Number within brackets is the percentage of patients remaining in sinus rhythm, correlated to total number of patients after that observation period.

sinus rhythm was maintained the following day (18, 34). On the other hand there was a very low frequency of relapses during the first 24 hours (Fig 1). Another explanation might be the time when maintenance therapy was started. Rossi and Lown (36) have recently reported on the benefits of starting quinidine prior to the shock. Others believe as we do that there might be more arrhythmias at conversion if quinidine is already administered (1, 6). Thus it is possible that the first method increases the convertibility rate but causes more complicating arrhythmias.

With regard to immediate results some authors state that patients requiring high energy levels are less stable in sinus rhythm. Oram and Davies report on ten patients where 400 Ws were used and none was converted to sinus rhythm (28). Korsgren et al found that no patient requiring 300 Ws or more maintained sinus rhythm at the end of their follow up study (18). In our material 32 patients maintained sinus rhythm for more than one year and nine of these required 400 Ws. We believe that, if a first or second attempt at conversion is indicated then it is necessary to try also high energy levels (40). We do not consider that it is possible to predict the prognosis from the energy levels used for conversion.

In our material we have not found any sig

nificant difference in the energy required between groups which differ in etiology, age or sex. This is in accordance with the findings of many authors (18, 27, 34) but contrary to Oram and Davies who reported lower convertibility in patients over 50 years of age (28).

Weight seems to influence the energy level required: patients weighing more require more energy. But the difference in the weight classes investigated is not significant. We are not aware that this has been previously discussed. The explanation might be a decreased myocardial current.

In the group with unsuccessful conversions there was a fairly high percentage of patients with arteriosclerotic heart disease. The group of failures however is not different from the whole group of patients with arteriosclerotic heart disease in age and heart volume but only in the duration of atrial fibrillation. There are more patients with a long duration in the failure group.

With regard to the long term results the group with arteriosclerotic heart disease does not differ in any essential respect from the groups with valvular disease. This confirms some earlier reports (8, 18, 27).

The group with mitral stenosis as the sole lesion is doing better than the groups with combined valvular lesions as might have been expected and as has also been previously demonstrated (5, 22, 25, 28). We have already mentioned the high frequency of relapse in operated mitral stenosis which is in accordance with some other studies (18, 31).

A tight mitral stenosis should not be converted prior to surgery (15, 18, 22). After mitral commissurotomy or other corrective surgery the countershock should not be administered until after several weeks (15, 27). With regard to the last statement no results are given in this report but this is supported by a considerable number of patients in our department of thoracic surgery who were given countershock in the immediate postoperative period. In nearly every case the patient relapsed into atrial fibrillation shortly afterwards.

In the group with treated hyperthyroidism combined with atrial fibrillation we had two failures out of four patients. In spite of the group being small the poor result is noticeable. Divergent results have been reported (11, 30, 32, 34).

As a whole the long term results after DC conversion have been disappointing and are identical with those from the quinidine era (38). At least somewhat better results could have been expected owing to the generally higher reversion rate and to the fact that quinidine sensitive patients could be treated. Some long term results from other studies are shown in Table V. Comparisons of this kind are naturally complicated by variations in selection of material and duration of follow up. The problem that still needs to be solved is how to achieve effective maintenance therapy to avoid recurrence of the arrhythmia. In this respect conditions are not better today than they were years ago. At present we do not know whether quinidine or other antiarrhythmic drugs are of any value in preventing relapse in cases of atrial fibrillation (2, 18, 28).

According to our experiences as well as those reported by other investigators some pertinent indications for conversion of atrial fibrillation might be proposed.

I In patients with atrial fibrillation of recent date or extending up to a duration of some years DC conversion should be attempted. All energy levels should be used if needed. Practically no age limit exists; this applies particularly to intractable cardiac decompensation.

After a relapse a new attempt is recommended if the first was successful and the patient maintained sinus rhythm sufficiently long to show that clinically his condition had undoubtedly improved. If the first attempt was unsuccessful according to the above definition a second attempt might be made but a better result this time is to be considered rather as an exception.

II In all patients with atrial fibrillation and episodes of embolization.

III In patients with atrial fibrillation combined with a disease that can be cured or improved e.g. by surgery no conversion should be performed prior to such cure or improvement. Consequently a patient with acute myocardial infarction and atrial fibrillation should not be converted until the infarction is healed. A hyperthyroid patient should not be converted before becoming euthyroid. In patients with multivalvular disease without tight mitral stenosis on rare occasions conversion prior to surgery might be indicated in an attempt to improve the patient's condition.

for the surgical procedure. Another exception might be atrial fibrillation occurring in the course of an acute myocardial infarction where some authorities believe that immediate conversion is indicated.

IV After corrective surgery of any heart lesion atrial fibrillation should not be converted until at least 6-8 weeks postoperatively.

There are few absolute contraindications for its use today and these seem to be rather universal (a) Digitalis intoxication or arrhythmias due to digitalis. It is possible that quinidine intoxication also should be included here (1-31). (b) Atrial fibrillation with complete heart block where no hemodynamic improvement is possible by means of conversion. (c) Patients with a recent thromboembolic episode should not be converted before anticoagulant therapy has been applied. (d) Tight mitral stenosis.

Among more relative contraindications can be listed longstanding atrial fibrillation, severe mitral regurgitation, electrolyte imbalance (especially hypopotassemia) and finally atrial fibrillation in elderly patients without symptoms.

CONCLUSION

During a 4 year period (August 1963 to September 1967) 181 patients with atrial fibrillation were treated with DC shock. The convertibility rate was 81%. The long term results are presented. In the total material only 42% maintained sinus rhythm 3 months after conversion and 22% after one year in spite of prophylactic quinidine medication in most cases. The long term results are related to the duration of atrial fibrillation before conversion whereas age and heart volume do not influence the prognosis to any great extent.

The number of shocks given in various patient groups was studied. There was no significant difference with regard to age, sex and etiology. There seems to be a definite trend in regard to weight: patients weighing more required more energy though the difference was not statistically significant.

If conversion is indicated we believe that one should not refrain from using high energy levels. We found that among the patients who remained in sinus rhythm for one year after conversion as many as 28.1% needed one or more shocks with

an energy of 400 Watt seconds. This is contrary to the findings of some authors who claim that to some extent prognosis might be predicted from the energy level used for conversion.

In many of our cases repeated conversions were performed on the same patient. It was an exception only when a later conversion gave a better long term result than the first attempt.

Finally the indications and contraindications for conversion are discussed on the basis of both our experience and that of other investigators.

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Table I. Cholesterol esters

Value in mg per 100 ml of plasma if not otherwise indicated

Sub ject	Sex/ Age	Total lipid	Type ^c	Cholesterol		Chol esterol/ phospho lipid ratio	Fatty acid composition value in per cent of total													
				Total	Ester ^d		12:0 ^e	14:0	14:1	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3	19:0		
				Cholesterol/																
O. N.	♀/33	820	II	318	108	1.0	—	0.5	Tr ^h	12.5	4.3	—	0.2	0.9	27.9	47.9	0.4	0.5	—	
		856	320	340	1.1	—	0.4	—	2.5	1.1	—	0.2	0.2	6.1	6.1	0.4	0	—		
		690	277	246	0.9	—	0.9	Tr	16.1	5.9	—	Tr	0.9	26.8	37.8	0.4	Tr	—		
K. L.	♀/48	972	II	350	380	1.0	—	0.7	Tr	15.3	6.8	—	Tr	0.8	29.0	42.5	Tr	Tr	—	
		816	279	277	0.9	—	0.5	—	8.0	3.3	—	—	0.3	14.6	26.6	Tr	Tr	—		
		798	297	274	0.9	—	Tr	—	8.7	3.3	—	—	0.6	24.1	40.7	Tr	Tr	—		
H. T.	♀/63	754	II	246	253	0.8	—	Tr	—	12.3	4.9	—	—	Tr	23.9	55.1	—	Tr	—	
		676		230	237	0.8	—	Tr	—	10.8	5.9	—	—	0.4	27.8	46.8	Tr	Tr	—	
		914		372	383	1.1	—	0.6	Tr	14.3	7.3	—	Tr	0.5	26.7	46.7	Tr	Tr	—	
A. F.	♂/55	554	II	236	230	1.2	—	0.8	Tr	13.4	5.7	—	Tr	0.5	9.7	46.1	Tr	Tr	—	
		358		190	239	0.9	1.8	1.4	Tr	11.6	8.3	—	—	Tr	25.1	46.4	Tr	—	—	
		570		178	247	0.9	Tr	—	—	10.3	5.4	—	—	Tr	24.8	47.5	Tr	Tr	—	
S. B.	♀/59	1284	II	475	497	1.1	—	0.8	Tr	11.4	5.8	—	Tr	0.9	20.6	34.7	0.5	Tr	—	
		708		258	311	1.2	—	—	—	10.4	5.0	—	—	1.0	31.2	46.9	Tr	Tr	—	
		696		194	309	0.8	—	—	—	11.7	5.3	—	—	Tr	22.2	47.1	—	Tr	—	
E. B.	♀/68	940	II	392	403	1.5	—	Tr	Tr	14.6	6.7	—	—	1.3	28.6	44.3	Tr	Tr	—	
		908		357	430	1.3	—	Tr	—	9.8	3.7	—	—	1.0	22.2	51.4	Tr	Tr	—	
		A		♂/32	1080	V	438	447	1.4	2.1	Tr	Tr	11.8	3.1	—	—	1.3	26.0	35.9	—
		878		375	373	1.4	—	0.8	Tr	15.5	7.5	—	Tr	1.9	27.4	36.0	—	Tr	—	
		740		248	292	1.2	—	Tr	Tr	10.4	4.4	—	0.3	0.9	22.1	30.2	—	1.8	—	

^a Normal value 13 women and 18 men ^b Standard deviation According to Fredrickson's system of phenol pills
^c hyperlipoproteinemias ^d Total amount of cholesterol esters ^e The cipher preceding indicates the number of C atoms
in the fatty acid. The cipher following indicates the number of double bonds in the molecule ^f Unidentified components are grouped according to their retention values (T_R value) related to the retention value for 18:0 (=1.0)

the patients had primary myxedema two had type thyroidism after strumectomy for toxic goiter. One patient had become hypothyroid while being treated with thiarnazole (NFTN) and another had acute strumitis.

The normal values for the different fatty acids were obtained from 19 men and 14 women aged 25-71 years (mean age 48 years) all euthyroid and with no known disease involving lipid metabolism.

Blood samples were taken after the patient had fasted for 12-14 hours. Potassium-ethylene diamine tetra acetic acid (K₂EDTA) was used as a stabilising agent. The blood samples were taken from both hospitalized pa-

tients and outpatients. There is no reason to suppose that the hospital diet was different from the patients' average food. After collecting the samples the plasma was immediately separated by centrifugation. If the examination was not carried out within a few hours the samples were stored in a refrigerator at +4°C.

Lipoprotein electrophoresis was performed on paper at room temperature in barbital buffer with ionic strength of 0.05 pH 8.6 containing 1% albumin (human) and 0.001 M EDTA (14) for 2 1/2 hours at 70 V (about 4 ma per strip). For staining Oil Red O was used according to Jencks and Durum (17). The lipoprotein

T_R 244- 756	T_R 283- 93	20.3 (7.0)	20.4 (2.1)	T_R 430- 485	20.5	T_R 65- 70	T_R 95-110 22.6	Remarks
—	—	0.6	4.5	—	—	—	4.7 ^a	
—	—	1.3	1.7	—	—	—	4.7 ^b	
0.8	5	—	2.8	—	—	—	7.8	Hypothyroid Strumectomy sequelae PBI ¹ 15 BMR 8
—	0.9	0.4	4.5	—	—	13.6	10.0	Treatment started PBI 1.7
—	—	0.7	10.4	—	—	—	Tr	Now euthyroid treated with desiccated thyroxine PBI 5.4 Se thyroxine 6.0
—	—	—	4.9	—	—	—	—	Primary myxedema. PBI 0.3 BMR 60-77
2.0	5.0	—	1.4	—	—	—	38.2	For 3 weeks treated with desiccated thyroxine 100 U a day PBI 0.9 BMR 91
—	—	—	4.6	Tr	—	—	20.0	For 2 months treated with desiccated thyroxine
—	—	—	3.8	—	—	—	Tr	For 4 months still hypothyroid BMR 84 PBI 1.4
—	—	—	3.1	—	—	—	5.1	Now nearly compensated PBI 3.7 BMR 111
—	—	—	3.9	—	—	—	—	Primary myxedema. PBI 3.7 Se thyroxine 1.7 T ₃ 5.6
—	—	—	3.8	—	—	—	Tr	Treated for 2 weeks with desiccated thyroxine PBI 3.2 T ₃ 6.1
—	—	—	—	—	—	—	—	Se thyroxine 0.9 BMR 81-85
—	—	—	4.4	1.0	—	—	—	Treated for 4 weeks with desiccated thyroxine BMR normal PBI 3.2
—	—	—	—	—	—	—	—	T ₃ 7.3 Se thyroxine 2.0
—	—	—	5.1	—	Tr	—	6.9	Treated for 10 weeks with desiccated thyroxine PBI 4.0 T ₃ 8.7
—	—	—	—	—	—	—	—	Se thyroxine 4.9 Euthyroid
—	—	0.9	4.6	Tr	—	Tr	0.3	Primary myxedema Se thyroxine 1.7
—	—	—	5.4	—	—	—	—	For 1 month treated with desiccated thyroxine Clinically compensated T ₃ 8.6 Se thyroxine 7.9
—	—	—	5.0	Tr	—	—	5.6	Strumectomy 0 years ago BMR 58 Se thyroxine 0.9 Iodine treated
—	—	—	6.2	Tr	—	—	7.5	Now treated with desiccated thyroxine for 2 months Se thyroxine 6.7
—	—	—	—	—	—	—	—	PBI 4.3
—	—	—	3.4	—	1.1	—	—	Hypothyroid after Thycazol treatment. PBI 2.2 BMR 86 Se thyroxine 3.1
—	—	—	5.0	1.6	—	—	5.2	Thycazol discontinued for 1 month PBI 3.8 Se thyroxine 6.3
—	—	—	6.6	—	—	—	13.1	Diabetes mellitus for 15 years Now acute strumitis. PBI 1.4 Se thyroxine 2.3
—	—	—	6.8	—	—	—	4.1	1 month later still hypothyroid -treatment PBI 1.0 Se thyroxine 10
—	—	3.1	6.1	0.3	—	—	0.0	T ₃ 10.0
—	—	—	—	—	—	—	—	2 months later still hypothyroid T ₃ 8.8 Se thyroxine 1.1

^a PBI = protein bound iodine 10 µg per 100 ml Normal values 3.5-7.5 µg per 100 ml ^b Se thyroxine = serum thyroxine in µg per 100 ml Normal values 4.5-13.5 µg per 100 ml ^c T = 1 labelled triiodothyronene uptake in the erythrocytes Normal value 6.5-13 ^d Tr = trace

pattern was classified as suggested by Fredrickson et al (9).

Plasma total cholesterol was determined according to Rundle (10). Plasma triglycerides were determined chemically (6) as well as by column chromatography described below. Total plasma lipid was estimated gravimetrically after Folch et al (8).

The lipid fractions were separated by silicic acid column chromatography (19) into three fractions. 1. Cholesterol esters, triglycerides, free cholesterol and free fatty acids and 3. phospholipids. The small amount of non-esterified fatty acids in the second fraction was not taken into consideration.

Gas liquid chromatography. To estimate the fatty acid

pattern of the three main lipid fractions the methylated fatty acids were subjected to gas liquid chromatography as described by Jestung and Bang (13) using a Pye Argon Chromatograph at 188°C with an ethylene glycol succinate column. Argon flow was 30 ml/min. Detector Strontium 90 beta ionisation.

Thin layer chromatography. In order to separate the different fatty acids according to their number of double bond in certain cases the fatty acid methyl esters were subjected to thin layer chromatography on a AgNO₃-enriched silica gel layer (17) prepared by using a 1:5 (w/v) solution of AgNO₃ instead of water in the preparation of the plates. The methylated fatty acids were applied to the plates in a band dissolved in petrol.

Table III Phospholipids

Value in mg per 100 ml of plasma if not otherwise indicated

Value in mg per 100 ml of plasma if not otherwise indicated

		Fatty acid composition value in per cent of total																	T_R	
Subject	Sex/Age	Phospho- lipids	1	0	14.0	14.1	16.0	16.1	17.0	17.1	18.0	18.1	18.2	18.3	0.0	0.3	0.4	T_R 4.30- 4.85	T_R 6.5- 7.0	T_R 9.5-11.0
E N	/32	308	—	—	0.1	0.1	30.0	0.8	0.4	0.1	17.2	15.4	0.9	0.5	Tr	2.0	8.1	—	—	3.0 ^a
		785	—	0.1	0.1	4.0	0.8	0.2	0.5	—	9	7	3.9	0.3	—	1.0	1.9	—	—	1.6
		244	—	Tr	Tr	8.1	—	—	—	—	16.6	15.5	6.5	0.5	Tr	3	9.5	—	—	6.4
			—	—	—	14.1	Tr	—	—	—	14.2	17.5	11.1	1.0	0.4	5.1	16.9	—	Tr	9
A I	+/48	343	—	—	0.2	0.4	17.0	0.0	1.1	—	14.4	15.8	2.1	0.5	Tr	5.3	11.5	—	—	3.5
		333	—	—	—	23.5	—	Tr	—	—	21.8	20.5	24.9	Tr	—	2.2	7.1	—	—	2.2
		333	—	—	—	21.3	Tr	Tr	—	—	18.0	0.2	3.0	0.5	Tr	1.1	9.6	—	—	4.0
		326	—	—	Tr	5.1	Tr	—	—	—	19.0	17.6	—	—	Tr	1.1	8.0	—	—	2.2
I I T	9/63	321	—	Tr	Tr	0.5	—	—	—	—	12.1	25.9	2.8	Tr	—	1.1	7.1	—	—	2.2
		306	—	—	—	10.4	—	—	—	—	18.7	12.1	4.2	—	Tr	2.0	5.6	—	—	—
		334	—	0.3	0.7	0.4	2.3	Tr	—	—	16.6	18.1	24.9	0.6	Tr	2.4	7.5	—	Tr	4.0
		194	—	0.4	0.5	2.1	—	—	—	—	17.0	10.5	2.1	Tr	—	3.6	6.0	—	—	Tr
A I	5/55	711	—	Tr	Tr	35.2	—	—	—	—	14.3	17.9	21.1	—	Tr	1.8	6.7	—	—	2.1
		188	—	Tr	Tr	24.0	—	—	—	—	16.3	18.4	11.6	Tr	Tr	3.1	7.8	—	Tr	4.4
		443	—	Tr	Tr	6.2	—	Tr	Tr	Tr	0.2	19.5	23.6	Tr	Tr	0.7	9.8	—	Tr	Tr
		219	—	Tr	0.4	24.2	Tr	—	—	—	15.3	16.3	18.3	0.4	Tr	6.2	13.4	—	Tr	7.5
S D	/53	447	—	Tr	Tr	28.8	—	Tr	Tr	—	13.6	16.6	15.1	Tr	Tr	1.2	8.3	Tr	—	3.5
		251	—	—	—	24.4	—	Tr	Tr	—	18.5	17	5.7	Tr	—	2.8	11.2	Tr	—	—
		219	Tr	Tr	Tr	25	—	—	—	—	15.7	0.8	21.1	—	Tr	1.5	8.3	1.2	—	5.4
		761	—	Tr	—	0.1	Tr	—	—	—	11.3	16.6	5.1	—	Tr	Tr	10.8	1.1	—	4.1
A A	5/1	100	—	Tr	Tr	21.6	—	Tr	Tr	—	18.2	18.0	21.7	Tr	Tr	2.1	10.7	—	—	5.1
		215	—	Tr	Tr	21.8	—	—	—	—	17.0	17.4	27.4	—	0.6	1.8	11.0	Tr	—	2.7
		217	—	—	—	6.7	—	Tr	Tr	Tr	18.3	16.2	21.1	—	0.6	1.4	11.1	—	—	4.0

Abbreviations as in Table I

Normal value 1.5 women and 0 men

0 Stand. dev. deviation

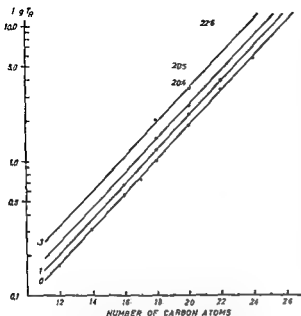


Fig 1 Logarithm of retention value (T_R) of methylated fatty acids plotted against the number of carbon atoms in the molecule obtained in assays with pure substances

benzene Benzene-diethyl ether 9:1 was used as a solvent. The chromatography was carried out by the sandwich technique. Visualisation was achieved by spraying at the borders with 2,7-dichlorofluorescein 0.2 w/v in 96% ethyl alcohol. The bands were then scraped off and re-extracted with diethyl ether and gas liquid chromatography was repeated.

RESULTS

The results are given in Tables I-III.

In all the hypothyroid patients moderate to marked hypercholesterolemia was observed. After treatment the plasma cholesterol level decreased. The esterification rate of cholesterol was within the normal range in the hypothyroid patients and no significant changes were noted during correction of the hypothyroid state. These results are in accordance with the findings of Furman et al (11).

All patients except one (a diabetic) had *tri* glyceride values within the normal range and no changes were observed when treatment was started.

The concentration of the phospholipids showed a nearly uniform decrease when the hypothyroid patients were treated.

In the plasma lipoprotein pattern by far the most common abnormality was an increase of the β lipoproteins which according to Fredrickson's system indicates type II hyperlipoproteinemia. After treatment these alterations disappeared. One

patient (the diabetic (K.A.)) did not belong to this type. His lipoprotein pattern seemed to belong to the combined type V hyperlipoproteinemia with a slight chylomicronemia as well as increased pre- β lipoproteins.

No changes seemed to occur in the α lipoproteins.

Fatty acid pattern In the fatty acid composition of the hypothyroid patients three types of abnormalities were observed: namely an abnormal accumulation of a component with a very high retention value (T_R), the occurrence of components with retention values not found in the 31 normal persons investigated and a relatively increased amount of arachidonic acid (C20:4) after the hyperthyroid state had been corrected.

The three abnormalities were not seen uniformly in all patients but formed a common pattern.

The first abnormality, the accumulation of a component with a very high retention value (9.5-11.0), occurred in the cholesterol esters sometimes in a very high degree and to a lesser degree in the triglycerides but not in the phospholipids. From several assays it was known that a fatty acid with a retention value around 10 is an indication of the fatty acid C22:6 $\Delta^4,7,10,13,16,19$ which normally appears in relatively small amounts in human plasma (where it can be synthesized from linolenic acid). However, as seen in Fig 1, the saturated fatty acid C26:0 also satisfies the retention value obtained. Normally C26:0 is not present in human plasma (22).

To identify this fatty acid a thin layer chromatography on AgNO_3 enriched silica gel layer was performed. Good separation according to the content of double bonds was obtained and in each case of increased concentration of the unidentified component it could be isolated from the saturated fatty acid group which moves in the solvent front of the chromatogram. This strongly supports the idea of a long chain saturated fatty acid viz C26:0. An example is shown in Fig 2. However, combination with a lesser amount of C22:6 cannot be excluded.

The second abnormality in the hypothyroid patients was the frequent occurrence of components with retention values not found in the normal subjects investigated. The components were present in all three lipid fractions and may be arranged in four groups. The first group contained fatty acid(s) with retention values rather close to 2.50.

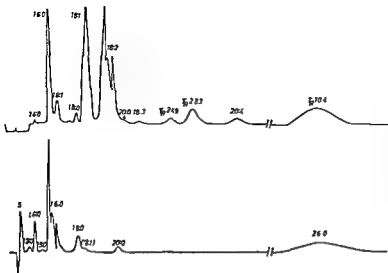


Fig Gas-liquid chromatogram of the methylated fatty acids from the cholesterol esters (patient O N) before and after isolation of the saturated fraction by thin layer chromatography. Except for a trace of C18:1 only saturated components are represented in the second curve. S means solvent.

The component occurred rather often but frequently in too small amounts for detection by gas liquid chromatography after attempts to isolate it by thin layer chromatography. Where this could be achieved the component was isolated from the saturated group. From Fig 1 the only possibility then seems to be C21:0.

The second group of fatty acids with retention values about 2.88 occurred twice (O N and K L) in the cholesterol esters in amounts which it was hoped would enable identification by gas liquid chromatography after separation by thin layer chromatography. Identification which satisfied our standard curve (Fig 1) was achieved in only one of the patients where the component was isolated among the monoenes. This seems to confirm the occurrence of the fatty acid C21:1.

The third group of fatty acids with retention values of 4.30–4.81 did not occur in amounts big enough for identification. C22:2 and C23:0 are possibilities according to Fig 1. This group is probably rather inhomogeneous.

The fourth group with the retention values of 5.5–7.0 was seen rather often and occurred twice (O N and K A) in sufficient amounts for identification by thin layer chromatography. It was found among the components with one double bond making C24:1 very probable.

The third abnormality of the fatty acid pattern was the relative increase in arachidonic acid after treatment of the hypothyroidism. In the cholesterol esters as well as in the phospholipids it occurred

in five out of the six patients who were followed to the euthyroid state.

The long-chain saturated fatty acid C22:0 could also be detected in our patients after its separation from C20:3 by thin layer chromatography. Our impression is that C22:0 does not occur in euthyroid patients on a normal diet but confirmatory results have not been obtained yet.

When the hypothyroidism was treated the above mentioned abnormalities disappeared on the whole. The changes in the phospholipids seemed slightly more resistant than those of the other lipid fractions.

DISCUSSION

No attempt was made to carry out a quantitative lipoprotein estimation. Paper lipoprotein electrophoresis was only used as a qualitative measure of the type of hyperlipoproteinemia. In trying to ascertain whether the decrease in the plasma phospholipids was due solely to the obvious decrease in the β lipoproteins or might also be due to a decrease in the phospholipid rich α lipoproteins (high density lipoproteins) a semi-quantitative estimation of this fraction was made from the strips.

Our impression is that the α lipoproteins do not undergo any great changes when the hypothyroid state is corrected. Opinions differ on this subject in the literature (3, 11, 18).

As mentioned earlier each of the three major

lipid groups in human plasma has its own characteristic fatty acid pattern which is relatively stable to dietary fatty loads. The human organism is capable of synthesizing saturated fatty acids *de novo* from acetyl CoA, malonyl CoA and NADPH and to elongate and desaturate *intrinsic* and *extrinsic* fatty acids by adding two carbon groups at a time (Acetyl CoA) or substrating two hydrogen atoms.

These possibilities are not unlimited. The *de novo* synthesis is limited to palmitic acid and stearic acid, whereas by elongation saturated fatty acids of a higher order can be formed. The desaturation system is not able to introduce a double bond beyond the 9th carbon atom in the molecule. Therefore unsaturated fatty acids with this property have to be added to the mammalian organism for synthesis of polyunsaturated fatty acids of higher order (7, 22).

The higher polyunsaturated fatty acids have a marked lowering effect on serum cholesterol. This effect is the greater the longer the chain and the larger the number of double bonds (21). Linoleic acid seems only to be active as a precursor of arachidonic acid (7) and the most active is C22:6 $\Delta 4, 7, 10, 13, 16, 19$. The long-chain saturated fatty acids seem to have the opposite effect, that of raising the serum cholesterol level.

The possible effect of the different fatty acids on the other plasma lipid groups does not appear to be at all clear.

The mechanism of the fatty acids with regard to lipid metabolism is not fully understood. One of the major problems is whether free or esterified cholesterol is the form in which it is oxidized. The first step in the conversion of cholesterol to bile acid is a hydroxylation of the steroid in the 7th position (2). By folding the molecule a polyunsaturated fatty acid in *cis* form in cholesterol ester is able to bring its second double bond close to the carbon 7 of cholesterol.

Although it is well established that feeding polyunsaturated dietary fats lowers the serum cholesterol concentration, attempts to ascertain if under these circumstances an increase in the production of bile acid takes place have not given uniform results (16). In hypothyroid patients decreased formation of bile acid has been observed which in turn increased when the patients were treated (2).

It was thought that a possible explanation of

the hyperlipidemia in hypothyrosis could be that lack of thyroid hormone changes the fatty acid synthesis and the rebuilding from the dietary fatty acids in such a way that the esters subsequently formed are of a type difficult for the human organism to metabolize.

The results obtained in this study appear to support this idea, as we found abnormal amounts of what seemed to be long chain saturated fatty acids possibly with a carbon number up to twenty-six. Long chain monoenes and maybe dienes of a type not seen in normal subjects were also found. Some of the results seem to indicate the possibility of the occurrence of long chain fatty acids with an uneven carbon number in the molecule.

A possible explanation of our findings is that the desaturation system depends more on the thyroid hormone than does the elongation system. When the saturated fatty acids are not desaturated their accumulation could possibly give rise to further elongation and result in a synthesis of long chain fatty acids not seen in euthyroid subjects.

CONCLUSION

The results are presented of a detailed investigation of the plasma lipids in seven patients with hypothyrosis.

The hypothesis seems to be confirmed that one of the causes of hyperlipidemia in hypothyrosis could be that thyroid hormone deficiency qualitatively changes the fatty acid synthesis and thus causes the formation of esters not easily metabolized by the organism as the normal esters.

This problem requires further investigation, more particularly a better identification of the abnormal fatty acids found in order to get a more precise picture of where the thyroid hormone acts in lipid metabolism.

ACKNOWLEDGEMENT

Aided by a grant from Statens almindelige Videnskabsfond.

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PREDNISONE GLUCOSE TOLERANCE AND SERUM LIPIDS IN SURVIVORS OF MYOCARDIAL INFARCTION

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Abstract Glucose tolerance has been examined by means of a prednisone glucose tolerance (PGT) test in 41 patients with clinically documented myocardial infarction 3 to 4 weeks after the attack and in age matched control subjects without clinical evidence of cardiovascular disease. In addition serum cholesterol and triglyceride levels have been determined and free fatty acid (FFA) concentrations measured prior to the PGT test and after administration of the glucose load.

Abnormal glucose tolerance curves were obtained in 53% of the patients 3 to 4 weeks after the infarction and in 25% of the controls. Seventeen patients were retested approximately six months after the first test and glucose tolerance in seven patients with initially abnormal PGT curves was found to be within normal limits while further impairment was observed in only four cases. A significant correlation between the PGT tests and serum cholesterol levels was found to exist only in patients over the age of 60 while no correlation could be observed between prednisone glucose tolerance and serum triglycerides or fasting levels of FFA. The mean decrease of FFA 1 hour after the administration of glucose was slightly less in the patients with myocardial infarction than in the controls, while a decrease of FFA to levels below 00 μ Eq/l was observed in approximately one third of the patients 1 and/or 3 hours after the glucose load.

It is concluded that the impairment of glucose tolerance which frequently can be observed after recent myocardial infarction is probably due only in a minority of cases to a latent diabetic condition and that other factors which are known to influence carbohydrate metabolism must be taken into consideration in explaining the observed disturbances of glucose homeostasis.

The association of myocardial infarction with manifest diabetes mellitus is well known and so is the usually transient glucosuria which may occur during the acute stage of infarction. Studies concerning oral glucose tolerance (1, 3, 4, 5, 7, 12, 17, 20, 21, 25, 32) or intravenous glucose tolerance (17, 22, 33) made during recent years in patients with coronary heart disease have

shown a surprisingly high incidence of impairment of glucose tolerance after recent myocardial infarction and have given rise to discussion concerning the possible significance of these abnormalities of carbohydrate metabolism in the pathogenesis of coronary atherosclerosis.

There seems however to be no agreement about the persistence of the observed impairment of glucose tolerance which is associated with myocardial infarction. A decrease in the incidence of abnormal glucose tolerance following a more prolonged interval after the acute stage of infarction has thus been observed during the course of some studies (7, 15, 25) while other investigators have expressed the view that the duration between infarction and testing does not significantly influence the results of the glucose tolerance tests (22, 33). Few studies on the other hand have included data which would allow conclusions to be drawn about the predictive value of glucose tolerance tests concerning the development of clinical diabetes in patients with coronary heart disease.

Steroid modified glucose tolerance tests which were first introduced by Fajans and Conn in 1954 (11) are regarded as a more sensitive means than unmodified glucose tolerance tests in detecting early abnormalities of glucose tolerance (6) and might therefore be expected to offer additional information concerning the incidence and nature of the early disturbances of carbohydrate metabolism in patients with myocardial infarction. Since only a few studies concerning steroid modified glucose tolerance in patients with coronary heart disease have been published to date, prednisone glucose tolerance (PGT) tests were performed in a group of hospitalized patients

Table 1 Number of positive PGT tests in different age groups of patients with myocardial infarction and in control subjects

Age groups	Myocardial infarction			Control group		
	No of subjects	Positive PGT tests	Negative PGT tests	No of subjects	Positive PGT tests	Negative PGT tests
< 50	18	7	11	13	4	9
50-59	15	8	7	9	2	7
60-69	8	7	1	9	~	7
Total	41 (100)	22 (53.6)	19 (46.4)	31 (100)	6 (25.8)	25 (74.2)

with recent myocardial infarction 3 to 4 weeks after the attack and repeated approximately six months after the initial test. Similar tests were performed in age matched control subjects with out clinical evidence of cardiovascular disease. In addition serum cholesterol and triglyceride levels were determined and free fatty acids (FFA) measured during the PGT tests.

MATERIAL AND METHODS

The study was performed in 41 patients with myocardial infarction (39 males and two females) who were hospitalized in the Department of Medicine of the Maria Hospital or in the IV Department of Medicine of the University of Helsinki. A complete evaluation including serial ECGs and determinations of SGOT was performed in all patients and only cases with clinically documented myocardial infarction were included. The average age of the patients was 57.4 years (range 36 to 69 y) and that of a control material consisting of 31 healthy volunteers or patients admitted to a medical ward for the evaluation of minor non vascular illnesses was 54.1 years (range 40 to 69 y). There were no significant differences in body weight between patients and controls. Subjects with obesity of 0 or more above the ideal weight were not included and individuals with previously known disturbances of carbohydrate metabolism or a family history of diabetes were likewise excluded from the study.

Glucose tolerance was determined after two or three days on a diet rich in carbohydrate by means of a PGT test which was performed in the patients with myocardial infarction during the stage of convalescence 3 to 4 weeks after the attack. In 17 patients retests were performed 4 to 10 months (average six months) after the initial test. The tests were performed according to the original cortisone glucose tolerance test described by Fajans and Conn (11) substituting 10 mg of prednisone for each 50 mg of cortisone. Persons weighing more than 7.5 kg received 12.5 mg of prednisone 8 hours and again 2 hours before a standard glucose tolerance test, which was performed by administering to the test

subjects 1 g of glucose per kg body weight. Capillary blood was withdrawn for the blood glucose determinations at 0, 1, 1 1/2, 2 and 3 hours after the administration of the glucose load and examined by a method for true blood glucose estimation (14).

A PGT test was regarded as positive according to the criteria for normality suggested by Conn and Fajans (6) if blood glucose values in excess of 160 mg% at 1 hour and blood glucose values in excess of 140 mg% at 2 hours were obtained. In order to facilitate comparison of the results of the PGT tests with serum lipid levels each glucose tolerance test was also expressed as an Area Index, an approximation of the area under the PGT curve which according to Vecchio et al (31) was calculated from the formula

$$\frac{A+B+C+D+E}{2}$$

where A is the fasting blood glucose value and B, C, D and E the subsequent values for the next two hours taken at half hour intervals. In this way a single numerical value is obtained to express the entire tolerance test, providing an index for the blood glucose level for the entire duration of the test.

Prior to the administration of the glucose load blood samples were obtained for the determination of serum lipids. Serum cholesterol was determined by the method of Pearson et al (19) serum triglycerides by a slightly modified procedure of Van Handel and Zilversmit (30) and FFA levels by the Dole procedure as modified by Trout et al (27). Blood samples for the determination of FFA were also obtained 1 hour and 3 hours after the glucose load simultaneously with the corresponding blood samples for the determination of blood glucose. (The analyses of serum triglycerides and the determinations of free fatty acids were carried out at the Lipid Research Laboratory of the Third Department of Medicine University of Helsinki).

RESULTS

Table 1 shows the number of positive and negative PGT tests obtained in patients with myocardial infarction 3 to 4 weeks after the attack

and the results of similar tests obtained in age matched controls. The material has been divided into three age groups according to decades. Positive PGT tests were in all three groups observed more frequently in the survivors of myocardial infarction than in the controls and an increase in the proportion of patients who had abnormal tests was seen with increasing age.

The results of the PGT tests obtained in the same group of patients and control subjects have in Fig. 1 been expressed as the mean PGT Area Indexes of the respective age groups. In the patients with myocardial infarction in all three groups the mean PGT Area Indexes were found to be higher than although not statistically different from the Area Indexes obtained in the respective control groups. The impairment of glucose tolerance was, however, found to be statistically significant ($P < 0.02$) when the three age groups were combined and the PGT Area Indexes obtained in all patients with myocardial infarction were compared with the corresponding indexes obtained in the control material.

The results of retests performed in 17 patients with myocardial infarction 4 to 10 months after the initial PGT test are shown in Fig. 2. It can

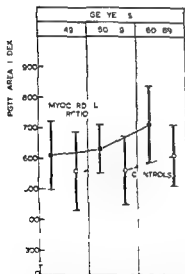


Fig. 1 Mean PGT test Area Indexes and standard deviations in three age groups of patients with myocardial infarction and in age-matched controls. The PGT test Area Indexes of the patients with myocardial infarction have been calculated from glucose tolerance tests performed three to four weeks after the acute attack.

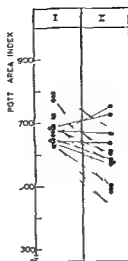


Fig. 2 PGT test Area Indexes calculated from glucose tolerance tests performed in patients with myocardial infarction 3 to 4 weeks after the acute attack (I) and approximately six months later (II).

be seen that at this time the PGT Area Indexes were in most cases lower than during the initial test performed in the same patients 3 to 4 weeks after the infarction. In seven patients originally abnormal PGT tests had at the time of retesting reverted to normal while some improvement of glucose tolerance was observed in six additional cases and PGT curves were found to be impaired in only four patients. There was no significant change in body weight of the patients at the time of retesting.

Mean levels and standard deviations of serum cholesterol and triglycerides in three age groups of patients 3 to 4 weeks after myocardial infarction are shown in Table II. Mean serum cholesterol values were found to be similar in all

Table II Mean levels and standard deviations of serum cholesterol and triglycerides in patients with myocardial infarction

	< 50	50-59	60-69 y
Cholesterol (mM)			
n	11	15	8
Mean	253	266	63
S.D.	111	69.6	58
Triglycerides (mg/dl)			
n	16	15	6
Mean	114	130	152
S.D.	43.9	51.3	59.1

Table III FFA levels before and after the oral administration of glucose in patients with myocardial infarction and in control subjects

	Mean age	FFA ($\mu\text{Eq/l}$)		
		0 h	1 h	3 h
Myocardial infarction	61.3			
n		25	25	25
Mean		642.3	397 (-38.2) ^a	300 (-53.3) ^a
s.d.		263.3	159.8	143.3
Control subjects	67.3			
n		27	21	22
Mean		634	374 (-41) ^a	377 (-48.4)
s.d.		157.7	158	242

^a Percent decrease of initial FFA levels after the administration of the glucose load

Table IV Distribution of positive and negative PGT tests in patients with myocardial infarction in relation to the amount of FFA decrease observed after the administration of glucose

PGT test	FFA levels 1 to 3 h after the oral administration of glucose		
	> 200 $\mu\text{Eq/l}$	< 200 $\mu\text{Eq/l}$	Total
Positive	14	8	22
Negative	13	5	18
Total	27	13	40

three age groups while serum triglyceride levels showed a tendency to increase with increasing

The relation between serum cholesterol and PGT tests performed in patients with myocardial infarction 3 to 4 weeks after the attack can be seen in Fig 3. A statistically significant correlation ($P < 0.05$) between serum cholesterol levels and the PGT Area Indexes was observed only in the patients over the age of 60 while no correlation was found to exist between serum triglycerides and prednisone glucose tolerance

Table III shows mean FFA levels before and after the administration of glucose to patients with recent myocardial infarction and the results of similar tests performed in control subjects. Mean fasting levels of FFA were found to be similar in patients and controls and no significant difference could be observed between the two groups in decrease of FFA after administration of the glucose load.

The number of patients with myocardial infarction in whom the FFA level after administra-

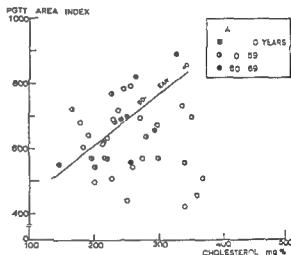


Fig 3 Relation of PGT test Area Indexes to serum cholesterol levels in patients with myocardial infarction

tion of the glucose load was less than 200 $\mu\text{Eq/l}$ is seen in Table IV. A decrease of FFA values below this level 1 and/or 3 hours after the glucose load was observed in 13 out of a total of 40 patients. Of these 13 patients eight had a positive PGT test while the distribution of positive and negative tests was found to be similar in patients in whom FFA levels after the administration of glucose did not decrease below 200 $\mu\text{Eq/l}$.

There was no correlation between the results of the PGT tests and fasting levels of FFA.

DISCUSSION

Prednisone glucose tolerance was found in the present study to be significantly impaired in patients with myocardial infarction 3 to 4 weeks

after the attack. The number of abnormal PGT tests was however not large compared to the number of abnormal curves obtained in patients with recent myocardial infarction after unmodified oral glucose tolerance tests which has been found to range from 41 up to 85 % (4 5 20 25 32). Reaven et al (21) on the other hand following the administration of cortisone observed only insignificant differences in glucose tolerance between patients with myocardial infarction and age matched control subjects. Steroid modified glucose tolerance tests would therefore not seem to be more sensitive than regular tests in detecting early disturbances of carbohydrate metabolism in patients with coronary heart disease.

The results obtained by different workers are however not directly comparable because the glucose tolerance tests have in many instances been carried out at different intervals after the infarction. The effect of ageing on glucose tolerance which in the present study was reflected by the greater number of abnormal PGT tests in patients with myocardial infarction with increasing age must moreover be taken into consideration in comparing glucose tolerance in survivors of myocardial infarction belonging to different age groups. In some studies (1 32) on the other hand control subjects have consisted of comparatively young healthy individuals in whom impairment of glucose tolerance is known to occur less frequently than in subjects belonging to the age groups in which coronary heart disease is prevalent.

During the course of the present study glucose tolerance was found to be improved in most cases subjected to follow up studies. This is in accordance with the results of Sowton (25) who made follow up studies of 30 patients with myocardial infarction and found abnormal oral glucose tolerance curves six months after the acute episode to be diminished from 73 to 43 % while three years later only 27 % of 15 patients had abnormal glucose tolerance. Similar observations have been made by Kimber (15) and by Datey et al (7) who found that the incidence of hyperglycemia in 57 cases of myocardial infarction was 70 % at the onset of the attack while abnormal glucose tolerance was found to be transient in 38 % of the cases. In contrast to the above mentioned reports Wahlberg (33) in his study of intravenous glucose tolerance in pa-

tients with myocardial infarction observed only an insignificant increase of the mean k values in patients who were retested within six months or 7 to 24 months after the infarction while an insignificant decrease of the mean k values was observed in patients retested more than 24 months later.

Only few studies have dealt with the predictive value of glucose tolerance tests concerning the development of clinical diabetes in patients with myocardial infarction (7 10 25 26). The incidence of diabetes three years after the acute episode was in the series of Sowton (25) 13 % while Datey et al (7) similarly observed that clinical diabetes had developed in approximately 14 % of their patients who had diabetic glucose tolerance curves shortly after the infarction. Eknerstrom (10) on the other hand found that 12 patients with initial hyperglycemia had normal glucose tolerance curves ten years after the acute attack.

It would thus seem probable that abnormalities of glucose tolerance after recent myocardial infarction are due only in a minority of patients to a latent diabetic condition and that other factors which are known to influence carbohydrate metabolism such as derangements of hypothalamic function caused by circulatory disturbances of the brain (20) increased secretion of adrenocortical steroids or an increased release of adrenaline (8) liver cell necrosis due to shock (25) low-carbohydrate diets and prolonged bed rest must also be taken into consideration in explaining the observed disturbances of glucose homeostasis.

The correlation between prednisone glucose tolerance and serum cholesterol or triglyceride concentrations was in the present study found to be poor except for the positive correlation between the PGT tests and serum cholesterol observed in the small group of patients over the age of 60. A lack of correlation between circulating levels of these serum lipids and glucose tolerance has similarly been observed in previous studies (1 21 25 33) and these observations together with the absence of correlation between serum immunoreactive insulin on the one hand and disturbances of carbohydrate or lipid metabolism on the other (17) would seem to oppose the view that the observed deviations from normal in the carbohydrate metabolism of pa-

tients with myocardial infarction may play a major role in the pathogenesis of the coronary artery disease.

Mean fasting levels of FFA 3 to 4 weeks after myocardial infarction were likewise not found in the present study to be different from normal. Other investigators have observed high FFA levels immediately after infarction (16) while Rifkind (22) in agreement with the present study found that FFA levels were not different from controls 3 weeks after infarction although the values were found to be high in patients with a more distant infarction. These differences in FFA levels measured at different points of time after the infarction are however not surprising since many factors including diet, physical activity, smoking and emotional stress are known to influence FFA concentrations.

The mean response of FFA levels to glucose which is usually less pronounced in diabetics than in normal subjects (2, 9, 13, 23) was during the present study found to be only slightly less pronounced in patients with myocardial infarction than in control subjects. Although a sluggish response of FFA to the oral administration of glucose has been observed by some workers in patients convalescing from myocardial infarction (24) Tzagournis et al. as in the present study recently found that the FFA response to glucose in young patients with coronary heart disease was virtually identical to that of normal subjects (28) while Cohen and Shafnir (5) observed in two thirds of their 43 patients three weeks after myocardial infarction a prompt and long lasting decrease of FFA after an oral glucose tolerance test. The adequate response of FFA to glucose which was observed in several cases in the presence of impaired glucose tolerance was attributed by these workers to a differential effectiveness of insulin action in adipose and non adipose tissue which according to Vallance-Owen and Ashton (29) could be explained by the appearance of insulin antagonists in patients with myocardial infarction.

It may be concluded that so far there is only little evidence that the often transient impairment of glucose tolerance which is frequently observed after recent myocardial infarction is correlated to alterations of lipid metabolism. Long term follow up studies in patients with coronary heart disease in whom signs of deranged

carbohydrate metabolism are detected would seem to offer a means of assessing the relative importance of different metabolic abnormalities as contributory factors in the pathogenesis of coronary atherosclerosis which according to current concepts (18, 28) can best be explained by the interaction of various atherogenetic factors over a period of many years.

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THE DEVELOPMENT OF CELLULAR HYPERSENSITIVITY IN MAN AFTER A PRIMARY IMMUNIZATION

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Abstract The development of cellular and humoral hypersensitivity was followed during a primary immunization with brucella bacteria in 15 persons. Both types of hypersensitivity were detected by *in vitro* techniques thereby enabling repeated recordings of the degree of hypersensitivity in each individual. Cellular hypersensitivity as measured by the leucocyte migration test developed in two phases separated by a shorter period of non-reactivity. The first phase was present as early as two days after the immunization. Humoral hypersensitivity measured as agglutinating antibodies followed the usual pattern with rising titers after a latent period of 6-8 days. Cellular hypersensitivity thus seemed to develop earlier than humoral hypersensitivity and apparently no correlation seemed to exist between these two parameters. The mechanism of the biphasic occurrence of cellular hypersensitivity especially the possible analogy with the so-called Jones-Mote's reactivity and the interrelationship between cellular and humoral hypersensitivity is discussed.

The primary immune response of an organism is considered to develop in two directions namely a cellular hypersensitive state and/or a humoral hypersensitive state. The distinction between these two hypersensitive states has been made mainly upon arbitrary criteria and has been of great value in defining and describing the characteristics of the two immunologic conditions. Depending upon the immunization procedure the applied antigen and the immunized organism the hypersensitivity will develop in one of the two directions or both. The question whether these two modes of immune reaction are unrelated or whether they represent the same basic immunologic process is still unsolved and subject to discussion.

The study of the relationship between these two types of hypersensitivity would among other things demand an exact simultaneous recording of both types following a primary antigenic

stimulus. In order to avoid interference with the immunologic reaction to be investigated *in vitro* techniques must be considered most suitable as they allow multiple measurements in the same individual during the course of immunization.

As to humoral hypersensitivity the technical basis of *in vitro* demonstration has been highly developed and the various steps of humoral antibody production are well clarified. In contrast little is known about the sequential development of cellular hypersensitivity. Some work has been done using the delayed intracutaneous reaction but this technique is not ideal because the antigen is introduced into the organism thereby altering the immunologic status to be examined.

The development of an *in vitro* technique for detecting cellular hypersensitivity using peripheral human leucocytes (24) offers an opportunity of recording the different steps of cellular hypersensitivity following a primary immune stimulus.

The purpose of the present study was to apply this technique together with the recording of humoral hypersensitivity in order to follow the development of both hypersensitive states during a primary immunization. *Brucella abortus* Bang was selected as antigen because this bacterium is able to elicit both types of hypersensitivity through the same immunization procedure.

MATERIAL AND METHODS

The material consisted of 15 persons with various intercurrent medical diseases. The patients were all in a good general condition and did not receive cortisone therapy or other drugs supposed to interfere with the immunologic response.

In order to ensure that the persons investigated indeed had a primary antigen stimulus, only brucella negative persons were examined. The criterion for brucella nega-

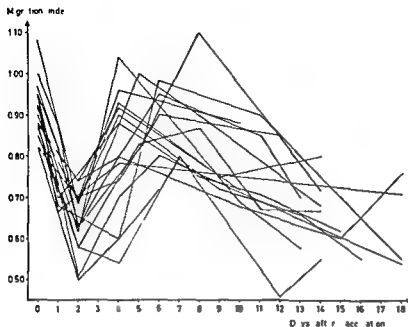


Fig 1 Variation of the migration indices in 15 persons after a primary immunization

tivity was a negative *in vitro* test of cellular hypersensitivity using brucella bacteria as antigen and no demonstrable titers of brucella agglutinins in the serum (see below).

As primary immune stimulus 1000 mill Brucella abortus Bang bacteria were injected subcutaneously in the thigh of each person.

Determination of cellular hypersensitivity and humoral antibodies was done at the same time in each individual as described below. All persons were investigated at time zero and subsequently every second and third day during the following 18 days.

Measurement of cellular hypersensitivity

In vitro detection of cellular hypersensitivity was formed with peripheral human leucocytes according to the technique described in detail in a previous paper (74). The principle of the method is based on the inhibitory effect of an antigen upon the migration of leucocytes *in vitro* in case the cells originate from an organism with cellular hypersensitivity to the same antigen. The migration of the leucocytes is measured in the presence and absence of Brucella bacteria. The action of antigen upon the cell migration is expressed in the so-called migration index M/M_0 , where M_0 represents the average migration area of the antigen containing cultures and M the average migration area of the control cultures. The migration index thus expresses the inhibition induced by the antigen in such a way that the more pronounced the inhibition the lower the migration index. The Brucella bacteria were of the same kind as used in the immunization.

The degree of migration inhibition has previously been shown to correlate with the degree of cellular hypersensitivity as expressed by the delayed intracutaneous reaction (25). According to these experiments a migration index of 0.78 was the borderline between brucella

positive and brucella negative observations. Consequently a migration index below 0.78 was the criterion of brucella hypersensitivity and persons were considered brucella negative if they had a migration index above 0.78.

Demonstration of humoral hypersensitivity

Circulating antibodies were determined by means of a brucella agglutination test. Serum dilutions from 1/10 to 1/1000 were placed in small test tubes and to each tube 1 ml of a Brucella abortus Bang antigen solution (0.2 vol %) was added (made available by Dr med vet H Bendtsen Statens Veterinær Serumlaboratorium Copenhagen). The tubes were incubated at 37°C for 18 hours. Agglutination titers were expressed as the highest final serum dilution that gave a 2 plus reaction on an arbitrary scale of zero through 4-plus.

RESULTS

Fig 1 shows the variations of the migration indices from 15 persons during the 18 days of the investigation. Each curve has a characteristic biphasic course starting with a decrease in migration indices during the first 2-4 days, a return to the original level about the 6th-8th day and then again a gradual decrease through the rest of the period. The uniformity of this biphasic course appears clearly from Fig 1 and seems to indicate a general variation in cellular hypersensitivity after the primary antigen stimulus.

Table I shows the migration indices at different times during the immunization together with the corresponding agglutinin titers. The mean

Table I Corresponding values of migration indices and antibody titers after a primary immunization

Pat no	Days after immunization													
	0		1-2		4-5		6-8		9-11		12-14		15-18	
	MI	AT	MI	AT	MI	AT	MI	AT	MI	AT	MI	AT	MI	AT
1	0.93	0	0.70	0	0.87	0	0.75	0	0.71	10			0.67	40
2	1.00	0	0.63	10	0.81	0			0.68	0			0.60	5
3	0.85	0	0.64	0	1.01	0	0.91	10			0.68		90	
4	0.98	0	0.64	0	0.81	0	1.10	0			0.71		10	
5	0.90	0	0.58	10	0.83	0	0.86	100	0.67	100	0.67	50		
6	0.87	0	0.74	0	0.91	0			0.73	10	0.78	20		
7	0.95	0	0.56	20	0.78	0	0.91	0					0.68	50
8	0.82	0	0.70	0	0.74	0	0.90	0			0.80	20		
9	0.90	0	0.50	0	0.70	0	0.80	0	0.70	10			0.54	30
10	1.08	0	0.68	—	0.90	10			0.7	0	0.66	20	0.55	50
11	0.95	0	0.69	10	0.84	0			0.75	10	0.84	50	0.80	100
12	0.85	0	0.70	40	0.58	0	0.99	0	0.89	10	0.80	10	0.54	20
13	0.91	0	0.62	0	1.04	0			0.69	50	0.58	30		
14	0.87	0	0.74	0	0.70	0	0.98	0	0.85	60	0.70	80		
15	0.87	0	0.59	0	0.50	0	0.80	10			0.46	90	0.55	100
Mean of MI	0.9±0.07		0.65±0.08		0.80±0.13		0.90±0.10		0.74±0.08		0.70±0.11		0.62±0.09	
Mean of change			-0.27±0.10		-0.11±0.16		+0.01±0.11		-0.19±0.13		-0.1±0.16		-0.33±0.11	

MI—migration index AT—antibody titer

values from each period show the aforementioned variation in the migration indices. Each group is statistically different from the population at time zero ($p < 0.001$) with the exception of the observations from the 6th–8th day which show the same distribution as the zero observations.

In order more generally to illustrate the individual changes the differences are calculated between the migration index at time zero and the migration indices on the various days after immunization of each person. The mean values of these differences called the means of change are also shown in Table I. It appears that these mean values follow the same pattern as indicated by the variations in the mean values of the migration indices.

The conclusion must be that under the chosen experimental circumstances cellular hypersensitivity develops during the first 1–2 days after immunization and reaches a maximum on the second day. The hypersensitivity then decreases and has disappeared on the 6th–8th day. From the ninth day the hypersensitivity reappears and reaches approximately the same level on the 15th–18th day as was obtained on the second day.

The development of humoral hypersensitivity

seems to follow another course. From Table I it can be seen that agglutinins do not appear until the 9th–11th day and show a gradual increase during the last part of the investigation period. Only in four patients (2, 7, 11 and 12) were small titers of agglutinin detectable on the second day, disappearing on the 4th–5th day.

In accordance with earlier observations (25) it can be seen that there is no correlation between the corresponding agglutinin titers and the migration indices.

DISCUSSION

From the present experiments at least three observations can be made: 1 cellular hypersensitivity develops at a very early stage in the immunization; 2 it appears in two phases; and 3 it develops apparently independently of humoral hypersensitivity as expressed by agglutinating antibodies.

1 Cellular hypersensitivity is most often considered to develop 10–15 days after the antigenic challenge as first described by Koch (12). The occurrence of shorter latent periods is however

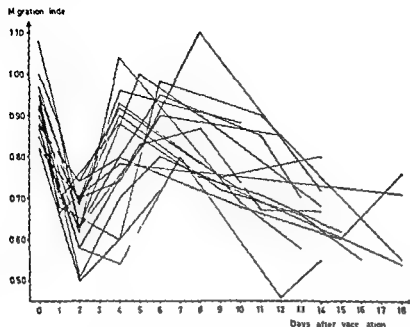


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The degree of migration inhibition has previously been shown to correlate with the degree of cellular hypersensitivity as expressed by the delayed intracutaneous reaction (5). According to these experiments a migration index of 0.78 was the borderline between brucella

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Table I shows the migration indices at different times during the immunization together with the corresponding agglutinin titers. The mean

As soon as this migration has been accomplished the individual is in a state of universal hypersensitivity. The diffusion of immunologically active cells has been shown to have a pronounced peak about 4-6 days after the immunization (11) and thereafter gradually to decrease to pre-immunization levels on the 10th day. If this piece of evidence is added to the well known fact that in a steady state of hypersensitivity immunocompetent cells are found in the circulation the conclusion might be that immunocompetent cells might be expected in high concentrations twice in the circulation perhaps interrupted by an intermediary phase with negligible amounts of cells. As the cells in the present experiments derived only from the peripheral blood it might be that the first phase of hypersensitivity represents cells on their way to settle down in more remote lymphatic centers where they will sensitize non-committed cells resulting in a universal state of hypersensitivity.

3 The last thing to be discussed is the relation between cellular and humoral hypersensitivity. During the first phase of cellular hypersensitivity detectable amounts of agglutinating antibodies are occasionally seen in very low titers. When the cellular hypersensitivity disappears circulating antibodies are found in rising titers. This is followed by increasing cellular hypersensitivity. As previously mentioned there is no correlation between the titers and the migration indices on the different days after immunization. This finding and the discrepancy in time sequence of the appearance of agglutinating antibodies and cellular hypersensitivity might indicate that the two types of hypersensitivity are produced by different immunologic mechanisms. This simple interpretation might however be incorrect as the sensitivity of the two parameters is probably very different and moreover on one side the reactivity of the cells is determined and on the other the result of cellular activity is the production of circulating antibodies. Thus experiments with the plaque forming cell technique have shown that antibody forming cells are detectable in the blood 1-2 days before the appearance of detectable amounts of circulating antibodies (8, 13, 17).

Other experiments with the plaque forming cell technique have shown that antibody forming cells develop in two peaks: the first represented by

cells forming 19 S antibodies and the second by cells forming 7 S antibodies occurring on the fourth and eleventh days respectively after the immunization (8, 29). Studies of the cell morphology connected with the immunoglobulin production have indicated that 19 S antibodies were probably associated with large basophilic cells and 7 S antibodies with plasma cells (21). No attempt was made to determine the degree of cellular hypersensitivity but according to the immunization procedure a certain state of cellular hypersensitivity might be expected to be present.

From the fact that antibody producing cells may appear in two peaks in the same way as cells responsible for cellular hypersensitivity it is possible that the events during the immunization may to some extent be similar and it would be tempting to advance the hypothesis that the first phase of cellular hypersensitivity is mediated by cells able to produce both 19 S antibodies and cellular hypersensitivity. The sporadic presence of low titers of agglutinating antibodies during this phase might be an indication of a temporary production of 19 S antibodies which are known to precede the production of 7 S antibodies in brucella hypersensitivity (30). Unfortunately the titers have been too low to determine the class of immunoglobulins in the present experiments.

In conclusion it may be said that the experiments have shown that cellular hypersensitivity to brucella bacteria develops in two phases. This is possibly an expression of a general immunologic phenomenon following any primary immunization. It is not possible from the presented data to give an explanation of the immunological mechanisms behind this biphasic curve or to elucidate much further the interrelationship between humoral and cellular hypersensitivity. The described experimental model together with other more advanced immunological techniques seem however to offer good possibilities of studying these very interesting problems more closely.

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EFFECT OF A NEW ADRENERGIC β BLOCKING AGENT H 56/28 ON NERVOUS HEART COMPLAINTS

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Abstract The effect of a new adrenergic β receptor blocking agent H 56/28 has been studied in fourteen patients with nervous heart complaints mainly palpitations. Organic heart disease and hyperthyroidism were excluded. The investigation was performed as a double blind test. During a first treatment period half of the patients received H 56/28 and the other half placebo-tablets; during the second period the treatment was reversed. The patients were also studied during repeated exercise tests. The heart rate during exercise was significantly reduced by H 56/28 and there was also a slight though not significant decrease of the systolic blood pressure. In addition to the immediate effect H 56/28 treatment seemed to have a more prolonged lowering effect on the heart rate. No side effects of the drug worth mentioning were seen. All but three patients preferred H 56/28 to placebo. The β blocker was especially effective in relieving palpitations. It is concluded that β blocking agents might be of value in treating patients with nervous heart complaints.

Several authors (1, 3, 4, 10, 11) have found β adrenergic blockade effective against nervous heart complaints. Not only the palpitations and other chest discomfort but also the anxiety were relieved. All these investigations were performed with propranolol. Another β receptor blocking agent with slightly different pharmacological properties H 56/28 (1-(*o*-allylphenoxy)-3-isopropylamino-2-propanol hydrochloride) has recently been described (8, 9, 12). In addition to its β blocking effect it is reported to have a weak β stimulating effect. In the present investigation trials were made with this new drug on an outpatient group with subjective cardiac symptoms where no signs of disease of the heart could be detected. The patients were studied both in rest and exercise.

MATERIAL AND METHODS

Fourteen outpatients have been investigated: five men aged 21 to 38 (mean 31) and nine women aged 17 to 57

(mean 31). The chief complaint of all the patients was palpitations including rapid and heavy action of the heart. As seen in Table I many of them also had a feeling of oppression or pain in the chest as well as nervousness, sweating, fatigue and dizziness. Pain in the chest and oppression were in all cases mild and not typical of coronary heart disease. None of the patients had hyperthyroidism.

The investigation was performed on a double blind basis (Fig. 1). At the first visit two exercise tolerance tests were carried out: one before (E_1) and another (E_2) one hour after oral intake of 80 mg of H 56/28. The patients rested between the tests. During the succeeding two to three weeks the patients received tablets marked A. After this period they returned for a second visit without having taken tablets for the last 24 hours. Again two exercise tests were performed before (E_1) and one hour after (E_2) taking their own tablets marked A. After a second period of two to three weeks when the patients were given tablets marked B they performed exercise tests in the same manner before (E_1) and one hour after (E_2) oral intake of their tablets marked B.

The tablets marked A given to group 1 (seven patients) contained H 56/28 (20 mg) of which they received 80 mg four times daily during the first treatment period and 80 mg before the second exercise test (E_2) at their second visit to the laboratory. During the second treatment period they received placebo. Group 2 (seven patients) received placebo during their first treatment period H 56/28 during the second period. Instead of the above mentioned dose the first four patients took 40 mg of H 56/28 four times daily and 40 mg of H 56/28 before the exercise tests.

The exercise tolerance tests were carried out with the subjects in the sitting position on an electrically braked bicycle ergometer (7). The first load was 200 kpm/min for women and 300 kpm/min for men. The work load was then increased by 200 kpm/min for women and by 300 kpm/min for men every 4th or 6th minute when the patient had reached a relative circulatory steady state, i.e. an increase in the heart rate of no more than four beats in two minutes.

During the exercise tests heart rate was estimated in the ECG every second minute. Blood pressure was measured indirectly in the arm. Before the investigation and at each succeeding visit the patients underwent routine

Table I Symptoms before and during the two treatment periods

	First visit	Symptoms					
		H 56/28 period			Placebo period		
		+	0	-	+	0	-
Palpitations	14	14	0	0	5	8	1
Pain in chest	9	5	4	0	3	5	1
Oppression	9	8	1	0	2	7	0
Breathlessness	6	5	1	0	3	3	0
Sweating	10	5	3	1	4	5	1
Tremor	5	6	0	0	2	4	0
Nervousness	11	6	5	0	3	7	1
Headache	7	6	1	0	3	3	1
Depression	7	7	0	0	5	2	0
Fatigue	10	6	4	0	4	6	0
Dizziness	9	6	3	0	4	4	1
Gastro-intestinal disturbances	4	1	1	2	2	1	1

+ = improved 0 = unchanged - = impaired

physical examination blood samples were drawn for analysis of ESR, Hb, WBC differential leucocyte count, thrombocytes, bilirubin, thymol reaction, alkaline phosphatase, glutamate oxalacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), lactate dehydrogenase isoenzyme determination (LDH-electrophoresis), creatinine and cholesterol. Urine samples were taken for analysis of protein and glucose and for microscopic examination. Before the investigation basal metabolic rate and proteinbound iodine were checked. X-ray controls of the heart and lungs were also made.

RESULTS

1 Subjective Symptoms

Almost throughout H 56/28 had a positive effect as compared with that of the placebo both regard-

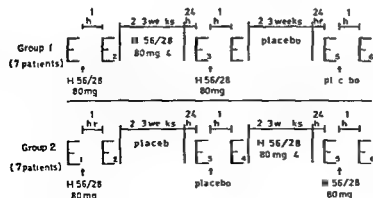
ing somatic and psychic symptoms. H 56/28 was especially effective in relieving palpitations, an effect which was seen in all the patients. Table I shows the effect of H 56/28 on the other symptoms.

In some cases the placebo had a positive effect. This was independent of whether or not the patients had received H 56/28 during a preceding period.

Two patients have reported gastrointestinal side effects, mainly nausea. One of them had however nausea also during the placebo period. After lowering the placebo dose from 4 to 2 tablets four times daily and the H 56/28 dose from 4 to 3 tablets four times daily, these side effects disappeared. Another patient had nausea after H 56/28 2 tablets four times daily when taking them before meals but did not feel sick when taking them after she had eaten. One patient complained of slight night sweating during treatment with H 56/28 which she had not experienced before the trial.

On asking the patients at the end of the trial which tablet (A or B) they preferred, all except three preferred H 56/28. One felt sick when taking H 56/28 before meals (see above) and therefore she preferred the placebo. She was given a low dosage of 2 tablets four times daily and reported that both H 56/28 and placebo had a good effect on her original subjective symptoms. Another patient after great hesitation stated that she found the placebo preferable as it had been more effective than H 56/28 on the heavy heart action. However, H 56/28 had

DESIGN OF EXPERIMENTS



E = exercise test

Fig 1 The investigation was performed as a double blind test. Group 1 received H 56/28 during the first and placebo during the second treatment period. In group 2 the treatment periods were reversed. Before and after these treatment periods two exercise tests (E) were made. Between these exercise tests H 56/28 or placebo was given.

had a good effect on her tremor vertigo and rapid heart action. A third patient who had previously been a moderate smoker could not decide which tablet was better. He felt better during the second treatment period when he got H 56/28 but thought that this was because he had begun to smoke again at the beginning of this period.

2 Physical Examination

Nine patients had a systolic apical murmur or a systolic murmur in the left second intercostal space always very discrete short and proto systolic. These murmurs have not been considered pathological. No changes in the physical condition of the heart were noted during the trial except for changes in heart rate and blood pressure which are described below.

On the first examination six patients had slightly moist and warm skin and four had finger tremor. After the placebo period these findings disappeared in half of these patients. After treatment with H 56/28 all the patients were normal in these respects. Apart from this the physical examination did not reveal any notable findings either before or during the trial.

3 Laboratory Tests

Before the trial was started one patient had GOT 49 and GPT 57 units and also a pathological LDH electrophoresis causing suspicion of liver damage. At controls after three and six weeks (placebo during the first period) GOT values were normal but GPT values were 76 and 75 units respectively and LDH-electrophoresis was unchanged. The other liver function tests and ESR were normal. Fine needle liver biopsy showed a normal cytological picture. The findings have been interpreted as remains of a subclinical hepatitis some time before the trial. It should be pointed out, that this patient had had his heart symptoms for several years which makes it rather improbable that the positive effect experienced during the H 56/28 period could be due to spontaneous regression. This patient also had a long history of orthostatic albuminuria which remained unchanged during the trial.

Another patient had an elevated GPT value 61 units after the H 56/28 period whereas it had been normal before the trial and after the placebo given during the first treatment period.

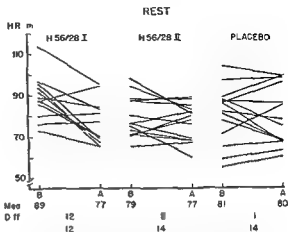


Fig. 2 Heart rate of each patient at rest before (B) and one hour after (A) H 56/28 or placebo. The examinations represented by the middle (H 56/28 II) and the right hand (placebo) part of the figure were preceded by treatment periods with the drug or placebo. Two patients were excluded at the first examination (H 56/28 I) for technical reasons.

GOT, LDH-electrophoresis, FSR and the other laboratory tests were however normal during the whole time. Three months later the patient still had GPT 58 units. The other liver function tests were normal. During these months he had been taking propranolol (20 mg four times daily). A few weeks later also GPT was normalised.

There were no other pathological findings during the trial.

X-ray examination of the heart and lungs was normal in all patients.

4 Heart Rate and Arterial Blood Pressure

Rest

The heart rate at rest before the first exercise test (E₁, Fig. 1) varied between 73 and 114 beats/min, mean 89/min (Fig. 2). Thus not all patients had tachycardia though all complained of palpitations. At the two exercise tests (E₂ and E₃) after the treatment periods the mean resting heart rate was about 10 beats/min lower than at the first time (E₁) and there was no difference between the heart rate at rest after the treatment period with H 56/28 and after that with the placebo.

One hour after H 56/28 was given for the first time (before the second exercise test E₂) the mean resting heart rate was 12 beats lower ($p < 0.02$) than before but the next time H 56/28 was taken there seemed to be no effect on the heart rate at

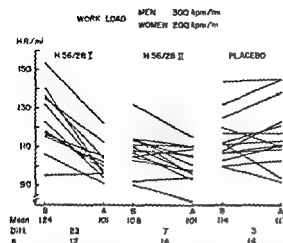


Fig 3 Heart rate during low work load before (B) and one hour after (A) H 56/28 or placebo. The examinations represented by the middle (H 56/28 II) and the right hand (placebo) part of the figure were preceded by treatment periods with the drug or placebo. Two patients were excluded at the first examination (H 56/28 I) for technical reasons.

rest. Nor was this the case after placebo (Figs 2 and 5). Thus it can be concluded that an exercise test does not seem to influence the resting heart rate one hour later. H 56/28 seems also to have little effect on the heart rate at rest when the patients are under more basal conditions (cf 8) which they probably were not the first time the drug was tested.

One patient had slightly elevated blood pressure (160–175/95) at rest while the others had normal pressures. At the two examinations after the treatment periods the mean resting systolic blood pressure was 7 mm Hg lower (Fig 7) and the diastolic pressure was virtually unchanged. The immediate effect of H 56/28 was on the two occasions when it was examined a slight though not significant decrease of the resting systolic blood pressure (Fig 7) while the diastolic blood pressure remained unchanged. The placebo changed neither the systolic nor the diastolic blood pressure which implies that the exercise test performed between the two measurements did not influence the blood pressure.

Exercise

Although many of the patients suffered from tiredness only a few of them had a somewhat low working capacity. One male (28 y) did not manage 900 kpm/min and three females (23, 43 and 52 y)

were not able to work on 600 kpm/min at a relatively steady state.

The main immediate effect of H 56/28 on the working capacity was that on several occasions the patients tried a higher working load than before. In four cases they were able to do this at steady state (cf 2, 3 and 5). After the treatment periods (E_2 , E_3) six patients were able to work at a higher load at steady state. In five of the patients however this could not be attributed to H 56/28 as it was achieved also after the placebo period which in these cases was the first treatment period. Two of these six patients reported that they had increased their physical activity during the treatment. In a few other cases after treatment with H 56/28 the patients tried a higher work load than at the first time but without reaching a steady state.

With only a few exceptions the heart rate during exercise was lowered after H 56/28 as has been reported for other β blocking drugs. The effect was more marked at higher work loads i.e. at higher heart rates (Figs 3, 4 and 5). The second time H 56/28 was tried during an exercise test i.e. after the H 56/28 treatment period the decrease of the exercise pulse was less noticeable. This might be due to the fact that the heart rate during the exercise test before the drug was administered was lower than at the first test (E_1). At the two exercise tests one hour after H 56/28

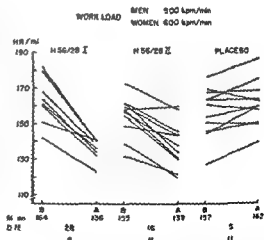


Fig 4 Heart rate during high work load before (B) and one hour after (A) H 56/28 or placebo. The examinations represented by the middle (H 56/28 II) and the right hand (placebo) part of the figure were preceded by treatment periods with the drug or placebo.

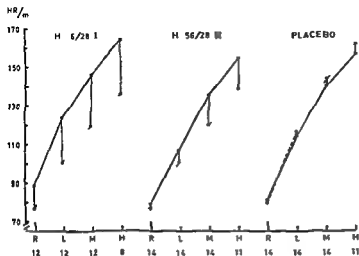


Fig 5 Mean heart rate at rest and during increasing work load before (—) and one hour after (---) H 56/28 and placebo. The examinations represented by the middle (H 56/28 II) and the right hand (placebo) part of the figure were preceded by treatment periods with the drug or placebo. R rest, L low (200 or 300 kpm/min), M medium (400 or 600 kpm/min), H high (600 or 900 kpm/min) work load.

the mean heart rates for each work load were almost identical. The mean heart rates at the two exercise tests after the placebo period were very similar, being only slightly higher the second time (+5 beats/min for the highest work load). Thus the first exercise influenced the second very little.

Fig 6 summarizes the "chronic effect of H 56/28 on the heart rate at rest and during exercise. The patients are divided into two groups, 1 and 2, according to the order of the treatment periods. The mean values are based on the first exercise test (E_1 , E_3 and E_5) at each of the three examinations.

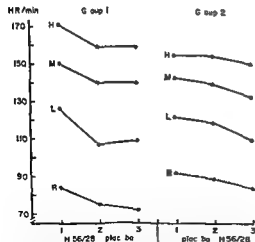


Fig 6 Mean heart rate at rest (R) and low (L), medium (M) and high (H) work load at the first exercise test (E_1 , E_3 and E_5) at each of the three examinations. The patients were divided into groups 1 and 2 according to the order of the treatment periods.

As can be seen, the entire decrease of the heart rate in group 1 was achieved during the H 56/28 period (except at rest), while in group 2 the lowering of the heart rate occurred in two steps: the second (corresponding to the H 56/28 period) being slightly bigger than the first. The difference between the two curves will be discussed later.

H 56/28 lowered not only the heart rate during exercise but also the systolic blood pressure (the diastolic pressure will not be discussed as its auscultatory measurement is rather unreliable during exercise). The first time the drug was taken, the effect seemed to be more marked; the higher the pressure, but this was not noticeable the second time (Fig 7). After placebo, the systolic blood pressure during exercise was virtually unchanged.

5 ECG

At rest, all ECGs were normal except in four patients where slightly depressed T waves were seen. In two of these, the ECGs were normalized one hour after H 56/28. In one of these patients, the ECG was normal also one hour after the placebo. In four cases with a normal ECG at rest, the amplitude of the T waves increased one hour after H 56/28. In one patient, this was seen also after the placebo.

During and shortly after exercise, three patients showed slight ST-T changes which were not regarded as a sign of coronary insufficiency. These changes were not noticeably influenced by H 56/28 or placebo.

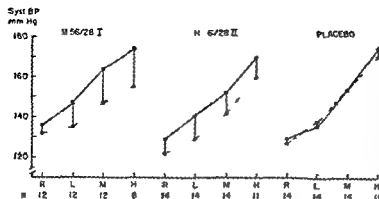


Fig 7 Mean values for systolic blood pressure at rest and during increasing work load before (—) and one hour after (---) H 56/28 and placebo. The examinations represented by the middle (H 56/28 II) and the right hand (placebo) part of the figure were preceded by treatment periods with the drug or placebo R rest L low (700 or 300 kpm/min) M medium (400 or 600 kpm/min) H high (600 or 900 kpm/min) work load

DISCUSSION

The patients studied in the present investigation must be considered a rather heterogeneous group. However, they all had one complaint in common: palpitations of the heart—the symptom which brought them to the doctor. Many of the patients also experienced pain in the chest and oppression which were invariably of vague and unspecific character. These symptoms like all the others gave the impression of being nervous or functional in origin. Coronary and other organic heart diseases as well as hyperthyroidism could in all probability be excluded by means of routine examinations, laboratory tests, X-ray and ECG at rest and during work. As only few of the patients had a low physical working capacity, the majority could not be said to represent the condition known as vasoregulatory asthenia (VA) (6), even if some of them probably belonged to this group. Furberg (5) has recently shown, however, that some changes of vasoregulation typical of VA patients may be found in subjects with a high physical working capacity. Bollinger et al (3) has also described a group of patients with neurovegetative resting tachycardia without impairment of working capacity.

Though the group of patients studied was not homogeneous, it was nonetheless fairly representative of the category of patients who seek medical advice for nervous heart complaints, especially palpitations. It should be noted that not all patients who complain of palpitations have continuous or sporadic true tachycardia, but rather define their suffering as a forceful or heavy action of the heart. Most of our patients had both a heavy and a rapid heart action. It seems quite reasonable that those who had tachycardia felt better

during the treatment with H 56/28 as one of the main effects of β blocking agents is reduction of the heart rate. It has recently been shown that H 56/28 is effective against sinus tachycardia (9). It was also well established in the present study that H 56/28 reduces the heart rate especially during exercise. The condition of those who complained of a predominantly heavy action of the heart was also improved, however, by H 56/28, which is interesting but less easily explained. It might be due to a negative inotropic effect of the drug. This property of H 56/28 is said to be weak in normal individuals (12), but might be of importance in this group of patients.

Concerning the subjective symptoms, all but three patients preferred H 56/28 to the placebo. The objective chronic effect of H 56/28 and the placebo also needs some consideration. The decrease in heart rate which was observed especially after the H 56/28 periods might be caused not only by this treatment but by other factors as well. The lowering of the heart rate in group 2 at rest and during exercise after the first period (placebo period) was most probably due to psychic factors, e.g. better knowledge of the experimental conditions or a feeling of being taken care of. This must imply that the decrease in heart rate in group 1 after the first period (H 56/28 period) was caused not only by H 56/28 but also by the psychic factors mentioned above. Similar psychic conditions probably existed at the third visit. This is also suggested by the curves for group 1 where there was no further decrease in heart rate (except at rest) after the placebo period.

Physical training during the treatment periods could also cause a decrease in heart rate, but this

factor is probably of less importance as all except two patients denied any increase in their physical activity during the treatment period. In these two cases it cannot of course be excluded that physical training may partly explain the decrease in heart rate during exercise.

Another explanation of the lower heart rate after the H 56/28 period might be that the last dosage of H 56/28 taken the previous day could still be effective. This seems rather improbable however as the heart rate was very similar also after the placebo period in group I.

Thus it can be said that after treatment with H 56/28 for a couple of weeks the effect on the heart rate at rest and during exercise persisted for some time and that probably this effect can not be completely explained by psychic factors and the result of physical training. How H 56/28 exerts its influence in this respect can only be however a matter of speculation but the possibility that during the treatment period a vicious circle has been broken should nevertheless be mentioned.

On the basis of the present investigation it seems reasonable to assume that β blocking agents can be valuable in the treatment of patients with nervous heart complaints.

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CLINICAL USE OF HIGH DOSES OF FUROSEMIDE (LASIX®) IN THE TREATMENT OF RESISTANT NEPHROTIC EDEMA

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Abstract Four cases of the nephrotic syndrome are reported in which furosemide was administered in doses higher than those usually prescribed. In three cases merely raising the dose of furosemide to levels up to 600 mg/day promoted a diuresis. In one patient furosemide in doses up to 1200 mg/day failed to produce a diuresis but the addition of a thiazide succeeded in producing one. Hypokalemia was seen in three patients and was treated by reduction of the drug dose, oral administration of potassium chloride and spironolactone. In cases of edema resistant to the usual doses of furosemide the use of higher doses alone or in combination with other diuretic agents may produce a satisfactory diuretic response.

Furosemide N-(2-furylmethyl)-4-chloro-5-sulfamoyl anthranilic acid is a relatively new diuretic that has been shown to be very effective in the treatment of edema resistant to other diuretic therapy (5, 7, 9, 14, 15). Patients are occasionally seen, however, who do not respond satisfactorily to combinations of furosemide and other diuretic agents in their usually recommended doses. In such patients the administration of much higher doses of furosemide (3, 5, 7, 14) even up to 1800 mg/day (5, 14) have been required to promote a diuresis.

This report concerns four patients with resistant edema secondary to the nephrotic syndrome in whom high doses of furosemide alone and in combination with other diuretics were administered when the usual doses failed to promote a diuresis.

MATERIAL AND METHODS

All four patients were adults and all showed membranous glomerulonephritis on renal biopsy. One patient had diabetes and one had lupus erythematosus. The other two had the nephrotic syndrome without associated diseases and with no history of a preceding acute glomerulonephritis. Furosemide was administered in tablet form, each

tablet containing 40 mg. The frequency of administration varied from 1 to 5 times daily. All patients were hospitalized throughout the study and all were restricted in sodium intake to 22 mEq/day. Observations made included body weight, urine volume, serum potassium, chloride and creatinine, blood urea nitrogen, blood uric acid, blood pH, serum bilirubin, serum glutamicoxaloacetic acid transaminase (SGOT), serum glutamic pyruvic acid transaminase (SGPT), serum alkaline phosphatase, hemoglobin, red cell count, differential and platelet count. Twenty-four hour collections of urine were analyzed for sodium, chloride, potassium and creatinine.

CASE REPORTS

Case 1

Diabetes and membranous glomerulonephritis (Fig. 1). A 28-year-old woman was first seen at the Renal Clinic in March 1965. She had had diabetes since June 1964 and had been maintained on about 38 units of lente insulin daily since that time. On this dose the fasting blood sugar remained around 125 mg/100 ml. Proteinuria was first noted in November 1964 and in January 1965 she began to note swelling of her legs. When seen at the Renal Clinic in March 1965 edema was noted in both legs up to the groin, and small pleural effusions were present bilaterally. The B.P. was 160/90 and the eye grounds were normal. The rest of the physical examination was unremarkable. The laboratory values are recorded in Table 1. Two LE clot tests and an excretory urogram were normal. A renal biopsy revealed membranous glomerulonephritis.

Over the next 3 months the patient received a combination of polythiazide 4 mg/day, spironolactone 25 mg three times daily, prednisone 5 mg three times daily, mannitol and albumin infusions, and two sequential courses of ACTH injections. On this treatment the edema decreased but never disappeared entirely. In June 1966 a marked increase in weight was noted, and did not respond to a combination of methylprednisolone 20 mg twice daily. Prednisolone was discontinued and azathioprine 100 mg daily was administered. Because of severe fluid retention scarification was performed but the patient continued to gain weight. Peritoneal dialysis was started on November 18, 1966, the patient's weight falling from 670 to 554 kg during this three-day procedure. After

Table I Summary of blood chemistry of the four cases

Case	1			2		3		4		Normal values
Date	3/65	11/66	1/67	10/65	10/66	1/67	8/66	1/67	11/66	
BUN	61	35	35	36	44	29	46	105	113	5-10 mg/100 ml
Serum creatinine	1.7	1.3	2.7	1.4	2.5	1.5	1.65	5.2	3.45	0.6-1.2 mg/100 ml
Creatinine clearance	39	14	13	51	44	45	54	14	20	70-150 ml/min
Hemoglobin	13.8	8.7	9.2	14.0	15.1	14.0	11.0	9.3	13.3	11.5-17.2 g/100 ml
Blood cholesterol	905	880	880	607	618	600	518	538	860	150-275 mg/100 ml
Serum albumin	1.5	0.7	1.0	1.8	1.8	1.7	2.2	2.3	2.7	4.8-5.8 g/100 ml
α 1 globulin	0.23	0.23	0.1	0.9	0.29	0.28	0.35	0.35	0.27	0.20-0.32 g/100 ml
α 2 globulin	1.38	1.86	1.50	0.75	0.63	0.67	0.4	0.47	0.84	0.32-0.54 g/100 ml
β globulin	0.92	0.98	1.33	0.73	0.73	0.74	0.82	0.86	0.71	0.43-0.85 g/100 ml
γ globulin	0.56	0.57	0.50	0.34	0.28	0.29	0.72	0.75	0.46	0.61-1.17 g/100 ml
Blood sugar	125	170	122	99	90	98	88	88	100	70-110 mg/100 ml
Serum Na	133	137	138	137	130	138	132	134	134	133-141 mEq/l
Serum K	3.8	3.8	3.4	3.8	3.7	2.7	4.4	4.4	4.4	3.3-4.4 mEq/l
Serum Cl	98	107	106	102	101	105	104	106	102	97-108 mEq/l

dialysis however the patient's weight gradually increased again despite furosemide 80 mg twice daily and spironolactone 25 mg three times daily. She was again hospitalized on January 11, 1967 and her hospital course can be seen in Fig. 1 and Table 1.

There was no diuretic response or loss of weight until the dose of furosemide reached 160 mg three times daily. At this point a slight diuresis was noted. When the dose was raised to 160 mg three times daily a marked diuresis occurred. The serum potassium was 3.4 mEq/l and fell to 2.9 mEq/l after one day on the high dose of furosemide despite the use of potassium supplements and spironolactone. Gradually over the next two weeks the serum potassium returned to normal. Little change was noted in the blood pH, BUN, serum creatinine or creati-

nine clearance. The blood sugar and insulin requirements during treatment did not change. The serum uric acid level was normal throughout the entire course. No changes in alkaline phosphatase, serum bilirubin, SGPT, hemoglobin, white cell or platelet counts were noted. Gradually over the next three months the patient was able to discontinue spironolactone and to reduce furosemide to 40 mg twice daily.

Case

Membranous glomerulonephritis (Fig. 2)

A 46-year-old man was first seen at the Renal Clinic in October 1965. In January 1964 he had noted a gradual onset of swelling in his legs and face. Slight proteinuria and mild hypertension were found at that time. He was treated with thiazide diuretics and the swelling disappeared in one week. In September 1965 he again began to note the gradual onset of swelling in his face and legs. When seen at the Renal Clinic in October 1965 moderate leg edema was found and pleural effusions were noted bilaterally. The BP was 130/140 and marked arteriolar spasm without hemorrhages or exudates was noted in the eye grounds. The rest of the physical examination was unremarkable. The laboratory values are recorded in Table 1. Two LE clot tests and an excretory urogram were normal. The patient was treated with prednisone 10 mg three times daily and chlorothalidon 100 mg daily. On this treatment his edema disappeared within four months and the medications were discontinued.

In September 1966 he again noted gradual onset of swelling in his legs and face. In October 1966 physical examination revealed marked leg edema and a BP of 170/110. A renal biopsy revealed membranous glomerulonephritis. The laboratory values are recorded in Table 1. Over the next two months despite treatment with furosemide 160 mg twice daily and spironolactone 5 mg four times daily his weight gradually rose from 67.0 to 75.9 kg.

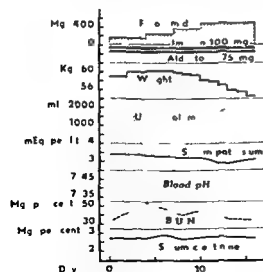


Fig. 1 Case 1 78-year-old woman. Diabetes and membranous glomerulonephritis.

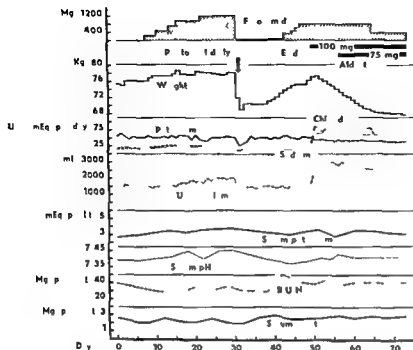


Fig 2 Case 2 56-year-old man
Membranous glomerulonephritis

In January 1967 the patient was hospitalized again and his course can be seen in Fig 2 and Table I. Over a period of two weeks the dose of furosemide was gradually increased to 400 mg three times daily but even after nine days of treatment with this dose no diuretic response occurred and severe fluid retention prompted peritoneal dialysis. The patient's weight fell from 78 l to 69.0 kg during this three day procedure. Gradually over the next four weeks however despite treatment with furosemide in doses up to 160 mg five times daily no diuretic response occurred. When hydrochlorothiazide 50 mg twice daily was added an immediate and brisk diuresis occurred. Within a few days a moderate hypokalemia developed with serum potassium falling to 2.8 mEq/l. The addition of spironolactone 75 mg three times daily raised the serum potassium to 3.5 mEq/l. Over the next three days and the potassium values thereafter stayed between 3.1 and 3.5 mEq/l. When the dose of furosemide was reduced to 160 mg five times daily an immediate decrease in sodium and chloride excretion was noted although there was little change in potassium excretion. Throughout the whole period little change was noted in the blood pH, BUN, serum creatinine and creatinine clearance. Both the fasting blood sugar and uric acid levels remained normal and unchanged throughout the study. No changes in the serum alkaline phosphatase, serum bilirubin, SGOT, SGPT, hemoglobin, white cell or platelet counts were noted. Gradually over the next three months the patient was able to discontinue spironolactone and the thiazides and to reduce the dose of furosemide to 40 mg twice daily.

Case 3

Lupus erythematosus and membranous glomerulonephritis (Fig 3 Table I)

A 67-year-old man was first seen at the Renal Clinic in June 1966. He had had discoid lupus erythematosus since 1933 and intermittent proteinuria since 1945. In April 1966 he noted the gradual onset of swelling in both legs. He was given chlorthalidone 100 mg daily but this was discontinued after seven weeks because it had no effect and made his skin lesions worse. From April until June 1966 the swelling gradually increased. When seen at the Renal Clinic at this time he was noted to have typical discoid lupus erythematosus lesions on his face and abdomen. Moderate edema was noted in both legs up to the groin. The BP was 175/95 and the eye grounds were normal. The rest of the physical examination was unremarkable. The laboratory values are recorded in Table I. Two LE clot tests were positive. An excretory urogram was normal. A renal biopsy revealed membranous glomerulonephritis.

The patient was given a 72 mEq Na diet but, because of his known sensitivity to medication no additional treatment was given. Over the next four months his edema steadily increased. He was seen again in November 1966 at which time it was noted that his creatinine clearance had decreased to 14 ml/min. He was given 80 mg of furosemide once daily and prednisone 10 mg twice daily. Despite this regimen the patient continued to gain weight and in January 1967 he was hospitalized. His course in the hospital can be seen in Fig 3 and Table I.

When the dose of furosemide was increased to 200 mg three times daily a moderate diuretic response occurred. Increasing the furosemide to 600 mg four times daily failed to increase the response further. When spironolactone was added in a dose of 5 mg four times daily an immediate increase in diuresis occurred. The addition of chlorothiazide at a late time increased the diuresis.

Table I Summary of blood chemistry of the four cases

Case	1		2		3		4		Normal values
Date	3/65	11/66	1/67	10/65	10/66	1/67	6/68	1/67	11/66
BUN	61	13	33	36	44	29	46	105	113
Serum creatinine	1.7	1.3	2.7	1.4	2.3	2.5	1.65	3.2	3.45
Creatinine clearance	39	14	13	52	44	45	54	14	20
Hemoglobin	13.8	8.7	9.2	14.0	15.1	14.0	11.0	9.3	13.3
Blood cholesterol	905	880	880	602	618	600	518	538	860
Serum albumin	1.5	0.7	1.0	1.8	1.6	1.7	2.2	2.3	2.7
α 1 globulin	0.3	0.23	0.3	0.29	0.9	0.28	0.35	0.35	0.2
α 2 globulin	1.38	1.86	1.50	0.75	0.63	0.67	0.47	0.47	0.84
β -globulin	0.97	0.98	1.33	0.73	0.73	0.74	0.8	0.86	0.71
γ globulin	0.56	0.52	0.50	0.34	0.8	0.29	0.7	0.75	0.46
Blood sugar	125	1.0	12	99	90	98	86	100	100
Serum Na	133	12	138	13	130	138	132	134	134
Serum K	3.8	1.8	3.4	3.8	3.7	2.7	4.4	4.4	4.4
Serum Cl	98	102	106	102	101	105	104	106	102

dialysis however the patient's weight gradually increased again despite furosemide 80 mg twice daily and spironolactone 5 mg three times daily. She was again hospitalized on January 11 1967 and her hospital course can be seen in Fig. 1 and Table I.

There was no diuretic response or loss of weight until the dose of furosemide reached 120 mg three times daily. At this point a slight diuresis was noted. When the dose was raised to 160 mg three times daily a marked diuresis occurred. The serum potassium was 3.4 mEq/l and fell to 2.9 mEq/l after one day on the high dose of furosemide despite the use of potassium supplements and spironolactone. Gradually over the next two weeks the serum potassium returned to normal. Little change was noted in the blood pH, BUN, serum creatinine or creati-

nine clearance. The blood sugar and insulin requirements during treatment did not change. The serum uric acid level was normal throughout the entire course. No changes in alkaline phosphatase, serum bilirubin, SGOT, SGPT, hemoglobin, white cell or platelet counts were noted. Gradually over the next three months the patient was able to discontinue spironolactone and to reduce furosemide to 40 mg twice daily.

Case 2

Membranous glomerulonephritis (Fig. 2)

A 56-year-old man was first seen at the Renal Clinic in October 1965. In January 1964 he had noted a gradual onset of swelling in his legs and face. Slight proteinuria and mild hypertension were found at that time. He was treated with thiazide diuretics and the swelling disappeared in one week. In September 1965 he again began to note the gradual onset of swelling in his face and legs. When seen at the Renal Clinic in October 1966 moderate leg edema was found and pleural effusions were noted bilaterally. The BP was 230/140 and marked arteriolar spasm without hemorrhages or exudates was noted in the eye grounds. The rest of the physical examination was unremarkable. The laboratory values are recorded in Table I. Two LE clot tests and an excretory urogram were normal. The patient was treated with prednisone 10 mg three times daily and chlorothalidon 100 mg daily. On this treatment his edema disappeared within four months and the medications were discontinued.

In September 1966 he again noted gradual onset of swelling in his legs and face. In October 1966 physical examination revealed marked leg edema and a BP of 170/10. A renal biopsy revealed membranous glomerulonephritis. The laboratory values are recorded in Table I. Over the next two months despite treatment with furosemide 160 mg twice daily and spironolactone 5 mg four times daily his weight gradually rose from 67.0 to 74.9 kg.

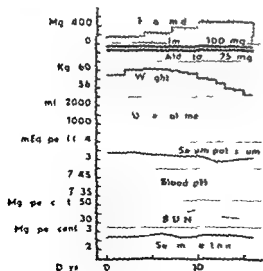


Fig. 1 Case 1 78-year-old woman. Diabetes and membranous glomerulonephritis.

nsing to 7 Ml Spironolactone in a dose of 25 mg three times daily was administered and furosemide was decreased and finally discontinued for four days. With improvement in the hypokalemic alkalosis the drug was again started in a dose of 80 mg twice daily. On this dose the patient's edema gradually increased over the next two months and only disappeared when the dose was raised to 170 mg twice daily. A marked improvement in the BUN, serum creatinine and creatinine clearance was noted during the course of therapy. The blood sugar and uric acid levels were normal and unchanged during the course of therapy. No changes in the alkaline phosphatase, serum bilirubin, SGOT, SGPT, hemoglobin, white cell or platelet counts were noted.

DISCUSSION

When no diuretic response occurs with the usual doses of a diuretic the tendency is to add another diuretic agent to the therapeutic program rather than to increase the dose of the original agent. Recent experience with furosemide and ethacrynic acid (5, 7, 14) indicates that merely raising the doses of these drugs may at times produce a diuresis when usual doses are unsuccessful. The usually recommended dose of furosemide is 40 to 200 mg/day. Doses of furosemide of 980 mg/day (7) and 1800 mg/day (5, 14) have been used on occasion and no toxic effects have been noted. In three of our four cases (cases 1, 3 and 4) 360, 600 and 360 mg respectively of furosemide per day were required before a diuretic response occurred. In case 2, however, there was no diuretic response with doses up to 1200 mg/day. The reason for this peculiar resistance of some patients to the administration of furosemide is not readily apparent. A poor diuretic response may be expected when there is a marked decrease in glomerular filtration rate (10). This is particularly the case when the creatinine clearance falls below 5 ml/min. In our cases, however, the creatinine clearances were well above this. The sodium reabsorbing tendency of the tubules is probably the major factor determining response to the diuretic, but factors governing this excessive reabsorption are obscure. Furosemide in the usually administered doses blocks sodium reabsorption primarily in the ascending limb of the loop of Henle (2, 6, 16, 17). In doses higher than ordinarily used it may also reduce sodium reabsorption in the proximal tubule (13). Possibly it is this combined action on the two sites that accounts for the diuresis when higher doses are used.

Combinations of furosemide with other diuretics may produce a diuretic response equal to or at times greater than their individual responses (3, 5, 14). In case 2 no diuresis occurred with 800 mg or 1200 mg of furosemide but the addition of a thiazide produced a profound diuresis. In case 3 a moderate diuresis occurred with 600 mg and 800 mg furosemide daily but the addition of spironolactone increased it markedly. A further increase was noted when a thiazide was added to the program.

In no patient was a reduction in renal function noted as judged by BUN, serum creatinine and creatinine clearance. In case 4 indeed a marked improvement in these parameters occurred. The reason for this is not entirely clear. It is known that furosemide increases renal blood flow and glomerular filtration (10, 11, 19). It has been suggested that this is accomplished by an inhibition of renin release from the juxtaglomerular apparatus (1). The diuresis itself may improve renal function by reducing renal interstitial edema thus alleviating tubular obstruction. Preliminary investigations suggest that the drug may play a useful role in the prevention and treatment of acute renal failure (1).

Toxicological evaluation of furosemide in animals has shown it to have a wide margin of safety (18). In our patients doses up to 1200 mg/day produced no toxic side effects. No reports of bone marrow aplasia or liver toxicity have been reported nor did these side effects occur in our patients.

Thrombocytopenia, skin rash, anorexia and diarrhea occasionally seen by others were not noted in our patients. Isolated reports exist of abnormal glucose tolerance tests precipitated by furosemide (8). No changes in fasting blood sugar were noted in our patients and there was no change in the insulin requirements of case 1 who had diabetes. The serum uric acid level not infrequently rises in patients taking furosemide but attacks of gout are exceedingly uncommon (5, 14). The serum uric acid concentrations did not change in our patients and attacks of gout did not occur.

Hypokalemia frequently seen with the administration of furosemide (5, 14) was seen in three cases but was extreme only in one (case 4). The loss of potassium and hydrogen is probably not caused by a direct effect of furosemide

on the tubules but by secondary aldosteronism. This complication may be partially or completely prevented by reducing the speed of diuresis. This is most effectively done by reduction of the dose or administration of the drug every second or third day. Potassium chloride supplements and administration of spironolactone or triamterene and the administration of ammonium chloride or arginine monochloride (rarely necessary) may all help in the prevention and treatment of this complication. The chloride ion is as important as the potassium ion in the correction of the alkalosis (12).

Intravascular fluid depletion with postural hypotension decreases renal function and shock may be seen if diuresis is excessive or rapid. One of our cases (case 4) developed mild postural hypotension but this disappeared spontaneously within a few days. The complication can frequently be prevented if the diuresis is not allowed to become excessive.

Occasionally patients will be seen who respond initially and later become resistant to the drug. In these patients correction of the hypokalemic alkalosis may improve the diuretic response. Drug resistance may also be countered by administering the drug every second or third day instead of daily. Finally increasing the dose of furosemide or adding other diuretics may again produce a diuresis. Three of our patients (cases 1, 2 and 4) actually required progressively less furosemide to remain free of edema.

Some workers (3) have found the diuresis produced by divided doses of furosemide to be greater than that produced by a single daily dose. Using both regimens we have found the diuretic response to be quite similar. A single morning dose however has the advantage of producing a diuresis during the day thus avoiding troublesome nocturia.

In our patients combinations of all the usual diuretics including furosemide in doses up to 200 mg/day, ACTH injections, steroids, azathioprine and intravenous albumin all failed to promote a diuresis. Scarification and peritoneal dialysis were resorted to in two patients to control edema. However three patients were relieved of their edema by merely raising the dose of furosemide and the fourth by a combination of furosemide with a thiazide. This suggests that in the patient with nephrotic edema who is resistant to

all diuretics including furosemide in the usually administered dose the dose of furosemide should be gradually increased (at a rate of 80–120 mg/day) until a diuretic response occurs. Doses up to 1800 mg/day may be needed (5, 14).

Another approach is to add other diuretic agents to the program when 200 mg of furosemide per day have failed to produce a diuresis. Spironolactone or triamterene would seem to be the agents of first choice because unlike thiazides, mercurials and carbonic anhydrase inhibitors they reduce the loss of both hydrogen and potassium in the urine. If no diuretic response occurs these other diuretic agents may then be added. If the edema still proves resistant the dose of furosemide may then be increased further.

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AN EVALUATION OF DC SHOCK TREATMENT OF ATRIAL ARRHYTHMIAS

Immediate Results and Complications in 437 Patients with Long term Results in the First 290 of these

Christopher Bjerkelund and Otto M. Ormring

From Medical Department B University Hospital Rikshospitalet Oslo Norway

Abstract A total of 1098 DC shocks were given in 57 attempts at reversion of permanent atrial arrhythmias in 437 patients. Of these 348 (80%) reverted to sinus rhythm during the first attempt.

Complications of the DC shock itself were infrequent. Ventricular arrhythmias and bradycardia occurred usually of short duration. Eight patients developed ventricular fibrillation and in one instance the episode was dramatic. Digitalis toxicity disposed patients to some of the ventricular arrhythmias. No deaths considered due to the DC shock were observed.

Thirteen patients (3%) developed an embolic episode 6 hours-6 days after reversion. Only two of these were amenable, the 28 patients who were on anticoagulant therapy as against 11 among the 209 who were not. Quinidine syncope caused by ventricular fibrillation was observed in eight patients.

The 290 patients first treated were followed up after the first attempt at reversion until relapse of the atrial arrhythmia or for a minimum of two years. After this period 73% of the patients treated were in sinus rhythm. This sinus rhythm attained was most stable in the patients with operated mitral stenosis, atrial septal defect and thyrotoxicosis that received treatment. The stability gradually decreased with increasing duration of the preceding arrhythmia but was little influenced by the heart size and even less by functional classification. Good long term results were obtained in individual patients with all etiological and anatomical types of heart disease.

Simple criteria can hardly be given for selection of patients who will benefit from reversion of atrial arrhythmia and careful evaluation of the individual patient is required.

Discovery of a new promising therapy is nowadays very soon followed by a wave of medical enthusiasm. Since the introduction of DC shock treatment of cardiac arrhythmias in 1962 (9) a vast literature on this subject has already been published (8, 11, 12, 15, 20).

Undoubtedly DC shock represents a very important advance in cardiological therapy. But there

is as already pointed out by Oram, equally no doubt that it has been used too enthusiastically (13). A more critical assessment of the indications for and value of this treatment is now gradually being established.

The purpose of this study is

1. to present the immediate results and complications of DC shock therapy in 437 patients with atrial arrhythmias.

2. to present the long term results in the first 290 of these patients who have all been followed up either until relapse of their atrial arrhythmia occurred or for a minimum of 24 months.

3. to see if more strict criteria can be established on the basis of our results and experiences for the selection of patients who will benefit from this treatment.

During these first years of the trial our aim has been to obtain as extensive experience as possible. Accordingly our indications have been most extensive: we have tried DC shock in all patients admitted to our department with permanent atrial arrhythmia. Our only contraindication was the presence of total A-V block or an imminent cardiac operation. In the last case at attempts to obtain electrical reversion were postponed until after surgery.

MATERIAL

A few remarks concerning the selection of the patients are appropriate. Rikshospitalet, the University Hospital, admits mainly patients referred from other hospitals all over the country. It has the only fully developed cardiac surgical center in Norway. As our department is working in close cooperation with this center we admit for hemodynamic studies and cardiological evaluation a much larger number of patients with serious acquired,

valvular diseases and congenital heart diseases, and a smaller number of patients with ischemic heart disease particularly acute myocardial infarction than is found in an unselected series of patients from our population. A considerable number of the patients with acquired valvular disease are at an advanced stage of their illness.

In this study are included all the patients admitted to the department with permanent atrial fibrillation atrial flutter and atrial tachycardia with block during the period from March 1 1964 to November 1 1967. During this period DC shock therapy was applied to all these patients, if not prevented by the presence of one of the two contraindications mentioned above. Thus a total of 1098 DC shocks were given in the course of 572 attempts to produce electrical reversion of atrial arrhythmia in 437 patients. In 454 of these attempts the atrial arrhythmia was reverted to sinus rhythm. In 112 of the 437 patients repeated attempts to obtain reversion were carried out. It should be stressed however that the data, which have been carefully analysed in this study and which will be presented are the immediate results of the first 437 attempts in respect of the total number of 437 patients, and the long term results of the first 790 attempts in the first 790 of these patients who were all followed up at least until relapse of the atrial arrhythmia or for a minimal period of two years.

METHODS

All our patients had been digitalized with digitoxin. This drug was omitted during the last 3-5 days before the procedure in all but the first 1.6 patients whose digitoxin was not discontinued.

In 347 of the patients quinidine was given usually in daily doses of 1.2 g. This treatment was started 3-5 days before the DC shock treatment, to determine the patient's tolerance and serum quinidine concentrations before reversion of the atrial arrhythmia was attempted. (In the first 93 patients quinidine was started either on the day or on the day before DC shock therapy was given.) If sinus rhythm was not attained quinidine was stopped. In all other cases it was continued indefinitely or until relapse of the atrial arrhythmia occurred except in a few patients where atrial fibrillation was due to a temporary cause (thyrotoxicosis operation for atrial septal defect). Only in 17 patients did quinidine in tolerance preclude the use of this drug. Four of these patients were treated with quinone in daily doses of 1.0 g. In the remaining 13 cases DC shock was given without any particular drug therapy to maintain sinus rhythm.

In 73 patients a β -adrenergic blocking agent (H 56 28) was tried as a matter of routine before and after electrical reversion was carried out. These patients are not included in the follow up material presented in this study. The result of this treatment will be published later (1).

In 2.8 patients, who were on long term anticoagulant therapy before admission to the department, this treatment was continued. In 709 patients, who were not on long term anticoagulant therapy anticoagulants were not given in connection with the attempt to obtain electrical reversion (2).

The DC shock therapy was applied in a fasting state under general anesthesia, with a short acting barbiturate (thiopental sodium). Pethidine (meperidine) 50 mg was administered subcutaneously was given as premedication 1 hour before operation.

A 12 lead ECG was recorded immediately before the procedure. A Corbin-Farnsworth synchronized DC defibrillator with large-diameter electrodes, was used. In approximately 1/3 of the attempts anterior electrodes were applied in the apical basal position. In the remaining 2/3 the anterior posterior placement was used.

If sinus rhythm was not established after the first shock of 100 ws and no signs of ventricular irritability (multiple ventricular premature beats or ventricular rhythm) developed further shocks were administered increasing the energy level to 200 300 and a maximum of 400 ws. More than four shocks were not given during a single attempt. All the patients were ventilated with oxygen before and after each shock.

After the procedure the patients were closely observed clinically and by means of an oscilloscope and ECG were repeatedly recorded until the patients were awake or for 5-10 minutes after the last shock was given. They were then transferred to the ward and further closely observed by the attending nurse. A new ECG was recorded 2-3 hours after the procedure. The treatment with digitalis was resumed for all patients. Quinidine (or H 56/28) was continued for all patients who were sent back to the ward in sinus rhythm or with AV nodal rhythm. Blood quinidine levels were determined on the day of the procedure and daily for the following few days.

A patient was regarded as having undergone reversion if sinus rhythm was attained and persisted until the patient was returned to the ward that is for at least 5 10 minutes or if the DC shock therapy resulted in AV nodal rhythm which after some minutes or hours changed into sinus rhythm. Patients who had only transient sinus rhythm lasting for only seconds or a few minutes, were not regarded as having undergone reversion.

Before beginning the study special forms were prepared for recording the appropriate data and the follow up observations for all the patients.

RESULTS

Table I gives a survey of the total material and the follow up material and the number and percentage of patients who reverted to sinus rhythm. Age and sex distribution and the primary results are almost the same in the follow up material as in the total material. Among the 437 patients treated with DC shock 348 (80%) primarily reverted to sinus rhythm.

It should be mentioned in this connection that among the 409 patients who were given quinidine before electrical reversion was attempted 62 (15%) reverted to sinus rhythm by means of this

Table I *Material*

Sex	Age (y)		No. of pats	Reverted to sinus rhythm	
	Mean	Range		No	Per cent
<i>Total material</i>					
Male	51.6	10-77	197	152	77
Female	51.3	18-81	240	196	82
Total	51.4	10-81	437	348	80
<i>Follow-up material</i>					
Male	51.1	10-77	133	102	77
Female	50.8	18-81	157	133	85
Total	50.9	10-81	290	235	81

therapy alone. Consequently DC shock was not given and these patients are not included in the present material.

The overall long term results in the follow up material are seen on the basal line of Tables II-VII and in Fig 1. Among the total number of 290 patients treated with DC shock 235 (81%) reverted to sinus rhythm. After 1 month 144 patients (50%) after 3 months 120 (41%) after 6 months 104 (36%) after 12 months 83 (29%) and after 24 months 67 (23%) of the patients treated were in sinus rhythm. It should be pointed out that the percentages are calculated from the total number of patients treated (not patients reverted). These results are similar to those reported by other investigators (4, 5, 6, 7, 11, 14, 20, 21).

Table II shows the primary results in the total material and the primary and long term results in the follow up material in relation to the type of atrial arrhythmias treated. The material includes patients with permanent atrial fibrillation as well as atrial flutter and atrial tachycardia with block. We wish to point out that the distinction between these three types of arrhythmia is not always very strict as transition from one type to another is frequently seen in the same patient. We have classified our patients according to the arrhythmia recorded on the ECG immediately before DC shock was given.

It is remarkable that atrial flutter which is one of the most difficult arrhythmias to terminate with quinidine and other drugs proves to be easily reverted with DC shock. It is evident from the table that both immediate and long term results in our material are somewhat better for patients

with atrial flutter than for those with atrial fibrillation. This is in agreement with results reported by other authors (8, 20).

The results observed in atrial tachycardia with block are also encouraging but the figures are small. It should be borne in mind that DC shock is contraindicated in this condition until digitalis toxicity has been excluded as the cause.

Table III shows the results in relation to etiology. Our material is dominated by patients with rheumatic valvular disease who represent 274 of the 437 patients i.e. almost $\frac{1}{3}$ of the cases. When considering the subgroups of patients with rheumatic etiology it is evident that the best primary and long term results were obtained in patients with operated mitral stenosis. Of these 89% primarily reverted to sinus rhythm and in the follow up material 20 of the 56 patients who had been treated were still in sinus rhythm.

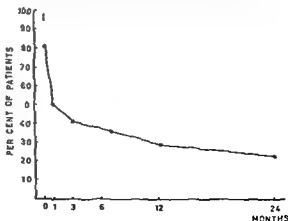


Fig 1 Percentage of the 290 patients first treated who were in sinus rhythm after different intervals of time.

Table II Results in relation to type of atrial arrhythmia

Type of arrhythmia	Total material			Follow up material						
	Total no of pats.	Reverted to SR		Total no of pats.	No of pats reverted to SR	Number of patients in SR after more than				
		No of pats	Per cent			1 mo	3 mo	6 mo	1 ¹ mo	4 mo.
Atrial fibrillation	372	290	78	251	200	115	98	82	65	51
Atrial flutter	46	41	89	29	26	21	17	17	14	12
Atrial tachy cardia with block	19	17	89	10	9	8	5	5	4	4
Total	437	348	80	290	235	144	110	104	83	67

SR = sinus rhythm.

more than two years after treatment. The good results in our material in patients with operated mitral stenosis are not in agreement with results reported by some investigators (7-21) but are more similar to those published by others (11-14).

Much less satisfactory are the results particularly the long term results for the patients with mitral regurgitation and also for the more complicated cases in which mitral valvular disease is combined with other valvular lesions. Of the 45 patients in both these categories in the follow up material treated with DC shock only about 10% (4 and 5 patients respectively) were in sinus rhythm

after more than two years. These results correspond with those obtained by other authors (8-11).

With regard to the non-rheumatic etiological categories listed in the table it is evident that the best immediate and follow up results were obtained in patients with atrial arrhythmia of thyrotoxic etiology. It should be emphasized that electrical reversion was not attempted in these patients before they had been successfully treated for their hyperthyroidism. The good results achieved in this etiological group are reported also by others (5).

The least satisfactory results were obtained in

Table III Results in relation to etiology

Etiology	Total material			Follow up material						
	Total no of pats.	Reverted to SR		Total no of pats	No of pats. reverted to SR	Number of patients in SR after more than				
		No of pats.	Per cent			1 mo	3 mo	6 mo	1 ¹ mo	4 mo
Rheumatic										
MS	2	18	82	15	12	6	5	4	3	3
MS op	84	75	89	56	50	33	30	8	24	0
MR, MR + MS	71	48	68	45	37	18	13	9	5	4
AS, AR	16	13	81	13	11	6	4	4	3	3
M + other	77	46	73	45	36	23	19	13	8	5
Constr. per	4	3	75	4	3	0				
Total	274	223	81	178	149	86	71	51	43	35
Art. scl. and/ or hypert	64	47	73	40	31	4	19	17	15	11
Congenital	33	26	79	21	16	12	10	10	9	
Thyrotoxic	13	11	85	10	9	7	7	7	6	6
"Lone fibr	27	19	70	22	15	8	7	7	5	-
Others	26	22	85	19	15	7	6	5	5	4
Total	437	348	80	290	235	144	110	104	83	67

SR = sinus rhythm. MS = mitral stenosis. MS op = operated mitral stenosis. MR = mitral regurgitation. AS = aortic stenosis. AR = aortic regurgitation. M + other = mitral and other valves affected. Constr. per = constrictive pericarditis.

Table IV Results in relation to relative heart volume

Relative heart volume (ml/m ²)	Total material			Follow up material						
	Total no of pats	Reverted to SR		Total no of pats.	No of pats reverted to SR	Number of patients in SR after more than				
		No of pats	Per cent			1 mo	3 mo	6 mo	12 mo	4 mo
<499	79	64	80	54	43	30	26	26	20	14
500-599	136	113	83	85	71	44	38	30	27	25
600-799	152	123	81	92	80	48	37	32	25	21
800-999	50	36	72	4	31	17	16	13	10	7
≥1000	17	11	65	14	9	5	3	3	1	0
Unknown	3	1		3	1	0				
Total	437	348	80	290	235	144	120	104	83	67

SR = sinus rhythm.

the patients with "long atrial fibrillation" without any other sign of heart disease. The reversion rate in this group was 70% and only two of the 22 patients treated were in sinus rhythm after more than two years. Hemodynamic studies have also shown that this category of patients benefits little from reversion of their atrial fibrillation (8).

In the group of patients with arteriosclerotic and/or hypertensive heart disease the primary results are less satisfactory but the follow up results are somewhat better than in the total group of patients with rheumatic valvular disease. Similar findings have been published by other investigators (6) but on the whole the results are variable (7, 11, 21).

In Table IV the results are seen in connection with the relative heart volume as determined by roentgenological examination. The size of the heart has little influence on the primary and long term results until pronounced enlargement is present. It is only when the relative heart volume is increased above 800 ml/m² that a considerable

deterioration in the results is demonstrated. More or less similar results have been reported by others (5, 7, 11, 14).

The results in relation to the functional classification of the patients at the time of reversion (according to the New York Heart Association) are given in Table V. Functional classes I and II are compared with III and IV. The primary and the long term results are hardly better in the first group. Morris et al. (11) who classified their patients in the same way found a more impressive difference. This may be due to a different selection of patients.

Much more marked is the relation of the results to the duration of the preceding arrhythmia as demonstrated in Table VI. The reversion rate decreases gradually with increasing duration of preceding arrhythmia, particularly when it has been present for more than two years. This influence is even more clearly shown in the long term results. Thus the stability of the attained sinus rhythm is also seen to decrease gradually with

Table V Results in relation to functional classification

Functional class	Total material			Follow up material						
	Total no of pats	Reverted to SR		Total no of pats	No of pats reverted to SR	Number of patients in SR after more than				
		No of pats	Per cent			1 mo	3 mo	6 mo	12 mo	24 mo
I and II	312	255	82	195	162	103	87	75	60	45
III and IV	125	93	74	95	73	41	33	29	21	12
Total	437	348	80	290	235	144	120	104	83	67

SR = sinus rhythm.

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Immediate Results and Complications in 437 Patients with Long term Results in the First 290 of these

Christopher Bjerkelund and Otto M Orning

From Medical Department B University Hospital Rikshospitalet Oslo Norway

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Complications of the DC shock itself were infrequent. Ventricular arrhythmias and bradycardia occurred usually of short duration. Eight patients developed ventricular fibrillation and in one instance the episode was dramatic. Digitalis toxicity disposed patients to some of the ventricular arrhythmias. No deaths considered due to the DC shock were observed.

Thirteen patients (3%) developed an embolic episode 6 hours-6 days after reversion. Only two of these were among the 278 patients who were on anticoagulant therapy as against 11 among the 209 who were not. Quinidine syncope caused by ventricular fibrillation was observed in eight patients.

The 290 patients first treated were followed up after the first attempt at reversion until relapse of the atrial arrhythmia or for a minimum of two years. After this period 3% of the patients treated were in sinus rhythm. The sinus rhythm attained was most stable in the patients with operated mitral stenosis, atrial septal defect and thyrotoxicosis that received treatment. The stability gradually decreased with increasing duration of the preceding arrhythmia but was little influenced by the heart size and even less by functional classification. Good long term results were obtained in individual patients with all etiological and anatomical types of heart disease.

Simple criteria can hardly be given for selection of patients who will benefit from reversion of atrial arrhythmia and careful evaluation of the individual patient is required.

Discovery of a new promising therapy is nowa days very soon followed by a wave of medical enthusiasm. Since the introduction of DC shock treatment of cardiac arrhythmias in 1962 (9) a vast literature on this subject has already been published (8, 11, 12, 14, 20).

Undoubtedly DC shock represents a very important advance in cardiological therapy. But there

is as already pointed out by Orning (11) no doubt that it has been used too early and too extensively. (13) A more critical assessment of the indications for and value of this treatment is gradually being established.

The purpose of this study is:

1. to present the immediate results and complications of DC shock therapy in 437 patients with atrial arrhythmias.

2. to present the long term results in 290 of these patients who have all been followed up either until relapse of their atrial arrhythmia occurred or for a minimum of 24 months.

3. to see if more strict criteria can be given on the basis of our results and experience for the selection of patients who will benefit from this treatment.

During these first years of the trial it has not been possible to obtain as extensive experience as possible. Accordingly our indications have not been as extensive. We have tried DC shock in patients admitted to our department with permanent atrial arrhythmia. Our only contraindication was the presence of total A-V block or an imminent cardiac operation. In the last 2 years we have attempted to obtain electrical reversion where postponed until after surgery.

MATERIAL

A few remarks concerning the selection of the patients are appropriate. Rikshospitalet, the University Hospital, admits mainly patients referred from other hospitals over the country. It has the only fully developed surgical center in Norway. As our department is in close cooperation with this center we admit patients from dynamometry studies and cardiological much larger number of patients with

valvular diseases and congenital heart diseases and a smaller number of patients with ischemic heart disease particularly acute myocardial infarction than is found in an unselected series of patients from our population. A considerable number of the patients with acquired valvular disease are at an advanced stage of their illness.

In this study are included all the patients admitted to the department with permanent atrial fibrillation, atrial flutter and atrial tachycardia with block during the period from March 1 1964 to November 1 1967. During this period DC shock therapy was applied to all these patients if not prevented by the presence of one of the two contraindications mentioned above. Thus, a total of 1098 DC shocks were given in the course of 577 attempts to produce electrical reversion of atrial arrhythmia in 437 patients. In 454 of these attempts the atrial arrhythmia was reverted to sinus rhythm. In 112 of the 437 patients repeated attempts to obtain reversion were carried out. It should be stressed however that the data which have been carefully analysed in this study and which will be presented are the immediate results of the first 437 attempts in respect of the total number of 437 patients, and the long term results of the first 290 attempts in the first 290 of these patients who were all followed up at least until relapse of the atrial arrhythmia or for a minimal period of two years.

METHODS

All our patients had been digitalized with digitoxin. This drug was omitted during the last 3-5 days before the procedure in all but the first 16 patients whose digitoxin was not discontinued.

In 447 of the patients quinidine was given usually in daily doses of 1 g. This treatment was started 3-5 days before the DC shock treatment, to determine the patient's tolerance and serum quinidine concentrations before reversion of the atrial arrhythmia was attempted. (In the first 93 patients quinidine was started either on the day or on the day before DC shock therapy was given.) If sinus rhythm was not attained quinidine was stopped. In all other cases it was continued indefinitely or until relapse of the atrial arrhythmia occurred except in a few patients where atrial fibrillation was due to a temporary cause (thyrotoxicosis, operation for atrial septal defect). Only in 17 patients did quinidine in tolerance preclude the use of this drug. Four of these patients were treated with quinine in daily doses of 1.0 g. In the remaining 13 cases DC shock was given without any particular drug therapy to maintain sinus rhythm.

In 73 patients a β -adrenergic blocking agent (H 46 29) was tried as a matter of routine before and after electrical reversion was carried out. These patients are not included in the follow up material presented in this study. The result of this treatment will be published later (1).

In 228 patients, who were on long-term anticoagulant therapy before admission to the department, this treatment was continued. In 209 patients, who were not on long-term anticoagulant therapy anticoagulants were not given in connection with the attempt to obtain electrical reversion (2).

The DC shock therapy was applied in a fasting state under general anesthesia, with a short acting barbiturate (thiopental sodium). Pethidine (meperidine) 40 mg was administered subcutaneously was given as premedication 1 hour before operation.

A 1st lead ECG was recorded immediately before the procedure. A Corbin Farnsworth synchronized DC defibrillator with large-diameter electrodes, was used. In approximately 1/3 of the attempts, anterior electrodes were applied in the apical basal position. In the remaining 2/3 the anterior posterior placement was used.

If sinus rhythm was not established after the first shock of 100 w, and no signs of ventricular irritability (multiple ventricular premature beats or ventricular rhythm) developed further shocks were administered increasing the energy level to 200-300 and a maximum of 400 w. More than four shocks were not given during a single attempt. All the patients were ventilated with oxygen before and after each shock.

After the procedure the patients were closely observed clinically and by means of an oscilloscope and ECG were repeatedly recorded until the patients were awake or for 5-10 minutes after the last shock was given. They were then transferred to the ward and further closely observed by the attending nurse. A new ECG was recorded 2-3 hours after the procedure. The treatment with digitalis was resumed for all patients. Quinidine (or H 46/8) was continued for all patients who were sent back to the ward in sinus rhythm or with A-V nodal rhythm. Blood quinidine levels were determined on the day of the procedure and daily for the following few days.

A patient was regarded as having undergone reversion if sinus rhythm was attained and persisted until the patient was returned to the ward that is for at least 5-10 minutes or if the DC shock therapy resulted in A-V nodal rhythm which after some minutes or hours, changed in to sinus rhythm. Patients who had only transient sinus rhythm lasting for only seconds or a few minutes, were not regarded as having undergone reversion.

Before beginning the study special forms were prepared for recording the appropriate data and the follow-up observations for all the patients.

RESULTS

Table I gives a survey of the total material and the follow-up material and the number and percentage of patients who reverted to sinus rhythm. Age and sex distribution and the primary results are almost the same in the follow-up material as in the total material. Among the 437 patients treated with DC shock 348 (80%) primarily reverted to sinus rhythm.

It should be mentioned in this connection that among the 409 patients who were given quinidine before electrical reversion was attempted 62 (15%) reverted to sinus rhythm by means of this

Table I *Material*

Sex	Age (y)		No of pats	Reverted to sinus rhythm	
	Mean	Range		No	Per cent
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Male	51.6	10-77	197	152	77
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Male	51.1	10-77	133	107	77
Female	50.8	18-81	157	133	85
Total	50.9	10-81	290	235	81

therapy alone. Consequently DC shock was not given and these patients are not included in the present material.

The overall long term results in the follow up material are seen on the basal line of Tables II-VII and in Fig. 1. Among the total number of 290 patients treated with DC shock 235 (81%) reverted to sinus rhythm. After 1 month 144 patients (50%) after 3 months 120 (41%) after 6 months 104 (36%) after 12 months 83 (29%) and after 24 months 67 (23%) of the patients treated were in sinus rhythm. It should be pointed out that the percentages are calculated from the total number of patients treated (not patients reverted). These results are similar to those reported by other investigators (4, 5, 6, 7, 11, 14, 20, 21).

Table II shows the primary results in the total material and the primary and long term results in the follow up material in relation to the type of atrial arrhythmias treated. The material includes patients with permanent atrial fibrillation as well as atrial flutter and atrial tachycardia with block. We wish to point out that the distinction between these three types of arrhythmia is not always very strict as transition from one type to another is frequently seen in the same patient. We have classified our patients according to the arrhythmia recorded on the ECG immediately before DC shock was given.

It is remarkable that atrial flutter which is one of the most difficult arrhythmias to terminate with quinidine and other drugs proves to be easily reverted with DC shock. It is evident from the table that both immediate and long term results in our material are somewhat better for patients

with atrial flutter than for those with atrial fibrillation. This is in agreement with results reported by other authors (8, 20).

The results observed in atrial tachycardia with block are also encouraging but the figures are small. It should be borne in mind that DC shock is contraindicated in this condition until digitalis toxicity has been excluded as the cause.

Table III shows the results in relation to etiology. Our material is dominated by patients with rheumatic valvular disease who represent 274 of the 437 patients i.e. almost $\frac{1}{3}$ of the cases. When considering the subgroups of patients with rheumatic etiology it is evident that the best primary and long term results were obtained in patients with operated mitral stenosis. Of these 89 primarily reverted to sinus rhythm and in the follow up material 20 of the 56 patients who had been treated were still in sinus rhythm.

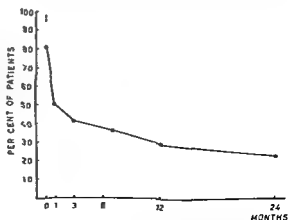


Fig. 1 Percentage of the 290 patients first treated who were in sinus rhythm after different intervals of time.

Table II Results in relation to type of atrial arrhythmia

Type of arrhythmia	Total material			Follow up material						
	Total no of pats.	Reverted to SR		Total no of pats.	No of pats reverted to SR	Number of patients in SR after more than				
		No of pats	Per cent			1 mo	3 mo	6 mo	12 mo	24 mo
Atrial fibrillation	372	290	78	251	200	115	98	8	65	51
Atrial flutter	46	41	89	29	26	21	17	17	14	1
Atrial tachy cardia with block	19	17	89	10	9	8	5	5	4	4
Total	437	348	80	290	235	144	120	104	83	67

SR = sinus rhythm

more than two years after treatment. The good results in our material in patients with operated mitral stenosis are not in agreement with results reported by some investigators (7-21) but are more similar to those published by others (11-14).

Much less satisfactory are the results particularly the long term results for the patients with mitral regurgitation and also for the more complicated cases in which mitral valvular disease is combined with other valvular lesions. Of the 45 patients in both these categories in the follow up material treated with DC shock, only about 10% (4 and 5 patients respectively) were in sinus rhythm

after more than two years. These results correspond with those obtained by other authors (8-11).

With regard to the non-rheumatic etiological categories listed in the table it is evident that the best immediate and follow up results were obtained in patients with atrial arrhythmia of thyrotoxic etiology. It should be emphasized that electrical reversion was not attempted in these patients before they had been successfully treated for their hyperthyroidism. The good results achieved in this etiological group are reported also by others (5).

The least satisfactory results were obtained in

Table III Results in relation to etiology

Et iology	Total material			Follow up material						
	Total no of pats	Reverted to SR		Total no of pats	No of pats reverted to SR	Number of patients in SR after more than				
		No of pats.	Per cent			1 mo	3 mo	6 mo	12 mo	4 mo
Rheumatic										
MS	2	18	81	15	12	5	5	4	3	3
MS op	84	75	89	56	50	33	30	28	4	0
MR MR MS	1	48	82	45	37	18	13	9	5	4
AS AR	16	13	81	13	11	8	4	4	3	3
M + other	7	56	73	45	36	23	19	13	8	5
Constr per	4	3		4	3	0				
Total	174	123	81	178	149	88	71	58	43	35
Art. scl. and or hypert	11	47	73	40	31	24	19	17	15	13
Congenital	33	26	79	21	16	12	10	10	9	7
Thyrotoxic	13	11	85	10	9	7	7	7	6	6
"Lone fibr"	7	19	70	2	15	8	7	7	5	
Others	26	22	85	19	11	7	6	5	5	4
Total	437	348	80	290	235	144	120	104	83	67

SR = sinus rhythm. MS = mitral stenosis. MS op = operated mitral stenosis. MR = mitral regurgitation. AS = aortic stenosis. AR = aortic regurgitation. M + other = mitral and other valves affected. Constrict per = constrictive pericarditis.

Table IV Results in relation to relative heart volume

Relative heart volume (ml/m)	Total material			Follow up material						
	Total no of pats	Reverted to SR		Total no of pats	No of pats reverted to SR	Number of patients in SR after more than				
		No of pats	Per cent			1 mo	3 mo	6 mo	12 mo	24 mo
<499	79	64	80	54	43	30	26	6	20	14
500-599	136	113	83	85	71	44	38	30	27	25
600-799	152	123	81	92	80	48	37	32	25	21
800-999	50	36	72	42	31	17	16	13	10	7
≥1000	17	11	65	14	9	5	3	3	1	0
Unknown	3	1		3	1	0				
Total	437	348	80	290	235	144	120	104	83	67

SR = sinus rhythm.

the patients with lone atrial fibrillation without any other sign of heart disease. The reversion rate in this group was 70% and only two of the 22 patients treated were in sinus rhythm after more than two years. Hemodynamic studies have also shown that this category of patients benefits little from reversion of their atrial fibrillation (8).

In the group of patients with atherosclerotic and/or hypertensive heart disease the primary results are less satisfactory but the follow up results are somewhat better than in the total group of patients with rheumatic valvular disease. Similar findings have been published by other investigators (6) but on the whole the results are variable (7, 11, 21).

In Table IV the results are seen in connection with the relative heart volume as determined by roentgenological examination. The size of the heart has little influence on the primary and long term results until pronounced enlargement is present. It is only when the relative heart volume is increased above 800 ml/m that a considerable

deterioration in the results is demonstrated. More or less similar results have been reported by others (5, 7, 11, 14).

The results in relation to the functional classification of the patients at the time of reversion (according to the New York Heart Association) are given in Table V. Functional classes I and II are compared with III and IV. The primary and the long term results are hardly better in the first group. Morris et al. (11) who classified their patients in the same way found a more impressive difference. This may be due to a different selection of patients.

Much more marked is the relation of the results to the duration of the preceding arrhythmia as demonstrated in Table VI. The reversion rate decreases gradually with increasing duration of preceding arrhythmia particularly when it has been present for more than two years. This influence is even more clearly shown in the long term results. Thus the stability of the attained sinus rhythm is also seen to decrease gradually with

Table V Results in relation to functional classification

Functional class	Total material			Follow up material						
	Total no of pats.	Reverted to SR		Total no of pats	No of pats reverted to SR	Number of patients in SR after more than				
		No of pats	Per cent			1 mo	3 mo	6 mo	12 mo	24 mo
I and II	312	55	82	195	162	103	87	75	60	45
III and IV	125	93	74	95	71	41	33	29	23	22
Total	437	348	80	290	235	144	120	104	83	67

SR = sinus rhythm.

Table VI Results in relation to duration of preceding arrhythmia

Duration of atrial arrhythmia (mo)	Total material			Follow up material						
	Total no of pats	Reverted to SR		Total no of pats	No of pats reverted to SR	Number of patients in SR after more than				
		No of pats	Per cent			1 mo	3 mo	6 mo	12 mo	24 mo
< 1	41	37	90	22	19	17	14	13	13	12
1-5	93	88	91	50	47	33	29	28	23	19
6-23	91	80	88	63	58	39	37	33	24	20
24-59	77	60	78	60	47	29	22	18	16	12
≥ 60	135	86	64	95	64	26	18	12	7	4
Total	437	348	80	290	235	144	120	104	83	67

SR = sinus rhythm.

increasing duration of the preceding arrhythmia. This result is in good agreement with those reported by others (7, 8, 11, 21) and also corresponds well with previous experience with quinidine induced reversion of atrial fibrillation (18, 19). It should be remembered however that atrial fibrillation of long duration is primarily seen in patients with serious heart disease, particularly rheumatic valvular disease at an advanced stage. But exceptions to this rule, especially lone fibrillation and atrial fibrillation in old age, are also well known.

In Table VII the long term results are presented in relation to the energy level required for the patients to revert to sinus rhythm. It is clearly demonstrated that early relapses are more frequent, observed in patients who needed a high energy level for reversion. In fact there is also a marked difference in the long term results between those patients who required 100 ws for reversion and those who needed 200 ws. Forty-four per cent of the first group and only 20% of the

second were in sinus rhythm after more than two years of observation. Similar findings have been reported (7).

Among the 67 patients who retained sinus rhythm for more than 24 months, 58 were still in sinus rhythm at their last control in the summer or autumn of 1967. They had then been observed for an average of 32.2 months per patient, varying between 24 and 45 months.

Of these 58 patients, 28 had rheumatic valvular disease, 13 arteriosclerotic heart disease, seven operated atrial septal defect, five thyrotoxicosis (treated), two primary myocardial disease, one operated constrictive pericarditis, and two lone atrial fibrillation. Among the 28 cases with rheumatic etiology, 19 were cases of operated mitral stenosis, two not operated mitral stenosis, one mitral regurgitation, three mitral and aortic valvular disease, and three aortic valvular disease. Thus it is evident that beneficial results of long duration can be obtained in patients with heart disease belonging to all etiological categories.

Table VII Follow up results in patients reverted to sinus rhythm in relation to energy level

Energy level (ws)	No of pats reverted to SR	Number of patients remaining in SR after more than				
		1 mo	3 mo	6 mo	12 mo	24 mo
100	87	61	53	49	43	39
200	102	62	49	38	27	20
300	21	11	10	9	7	5
400	18	10	8	8	6	3
Total	235	144	120	104	83	67

SR = sinus rhythm.

Deaths During Observation Period

Nine patients died during the observation period either before relapse of their atrial arrhythmia had been diagnosed or before the end of the observation period

Three died in the department

A 30 year-old woman who had been admitted at the terminal stage of chronic myocarditis died of serious congestive heart failure 12 days after reversion of an atrial tachycardia with block and clearly without any causative relationship to the DC shock therapy

A 67 year old woman with aortic stenosis and regurgitation and a history of angina pectoris and fits of dizziness who was suffering from serious congestive heart failure and 12 days previously had had a cerebral embolus died suddenly during the night 12 hours after the procedure. She was on quinidine. Autopsy did not disclose signs of fresh emboli or any other cause of sudden death

A man aged 57 with advanced mitral stenosis and regurgitation relative heart volume of 840 ml/m² and congestive failure died suddenly during the night 15 hours after reversion of his atrial fibrillation with DC shock. He was on quinidine. No autopsy.

Moreover three patients died suddenly at home 5, 14 and 16 days after the electrical reversion of their atrial fibrillation and clearly without any direct relation to this procedure. They were all on quinidine. No autopsy.

Finally three patients died at home most probably of heart disease 3, 10 and 25 months after the treatment with DC shock.

For all patients who died at home information was obtained from their physician and/or from their relatives. All the nine patients mentioned are included in the follow up material with an observation period from the reversion of their atrial arrhythmia until death.

It should be emphasized that the figures given in this paper are the results of the first attempt at electrical reversion in each patient. Our results are clearly less discouraging than those recently reported by Coelho et al (4) who found that only 5.9% of their 219 reversion patients remained in sinus rhythm for more than 24 months. Our figures cannot be compared with those reported by Morris et al (11) who presented the results of maintaining sinus rhythm with multiple reversion in the same patient. By this means they suc-

ceeded in maintaining sinus rhythm in the 20th month in 53% of their 108 patients.

Repeated attempts at reversion were however also tried in our patients. Thus in 112 of the patients 247 attempts were made varying between two and a maximum of ten attempts per patient. The result of these trials will not be presented here. It should be mentioned however, that in a few of these patients sinus rhythm was maintained for a considerably longer period after the second reversion than after the first.

Three of the patients in the present material were treated with DC shock because atrial arrhythmia developed while they were being treated continuously with implanted P synchronous pacemakers. In two of these patients the atrial arrhythmia was reverted to sinus rhythm judging from the P waves in the ECG but only in one patient was a synchronous pacemaker activity obtained. One patient maintained normal atrial activity for more than 25 months. The other two patients did not belong to the follow up material.

Complications

Four categories of complications are of interest in connection with DC shock therapy:

- 1 Complications related to the DC shock itself
- 2 Complications related to the reversion of the atrial arrhythmia
- 3 Complications related to the anesthesia
- 4 Complications related to the drug treatment given in connection with the attempt to obtain electrical reversion of the atrial arrhythmia

We will describe each of these categories separately.

1 The most common complications of the DC shock itself are disturbances in heart rhythm and conduction in most cases of benign character and short duration. Thus transient multiple ventricular premature beats frequently as bigeminal rhythm occurred in 120 of the 572 attempts made to obtain reversion in our patients. Ventricular tachycardia occurred in 13 patients but was of only 2-20 seconds duration and reverted spontaneously to sinus rhythm or to the original atrial arrhythmia in all the patients.

A more serious complication was ventricular fibrillation which occurred in eight patients. In four of these the arrhythmia was transient lasting

for only 2-25 seconds and reverted to sinus rhythm or the original arrhythmia without any special treatment. In the other four cases however the ventricular fibrillation was of longer duration namely 1 1/4, 4 and 20 minutes respectively. Ventilation with oxygen and external cardiac massage was immediately started and three of the patients were successfully defibrillated with one or two additional DC shocks.

The most dramatic episode was observed in the last patient, a 25 year old woman operated on three weeks previously for an atrial septal defect of primum type. In connection with the operation she developed atrial flutter. After the first DC shock of 100 ws ventricular fibrillation immediately developed and in spite of intubation and ventilation with oxygen external cardiac massage seven additional DC shocks and infusion of sodium bicarbonate persisted continuously until apparently spontaneously she reverted to sinus rhythm after 20 minutes. She had recovered completely on the following day.

Patients with advanced rheumatic valvular disease and large heart volumes had a special tendency to develop ventricular arrhythmias. Whether or not digoxin was discontinued some days before DC shock therapy was given could not be shown to influence the incidence of premature beats or ventricular arrhythmias after the procedure. In six of the patients in whom ventricular tachycardia or ventricular fibrillation developed ventricular premature beats were recorded immediately before DC shock. Premature beats especially if they increase in number after the first DC shock is a warning of impending ventricular arrhythmia if another shock is given. We believe that some of the cases of ventricular arrhythmia in our material developed because we did not fully take account of this warning during the first period of our trial.

Many patients temporarily developed nodal rhythm in response to DC shock treatment before a stable sinus rhythm was established. In most cases the nodal rhythm lasted only some seconds or a few minutes but a few patients remained in this ectopic rhythm for hours or even a day or two and then reverted to atrial fibrillation.

Five patients developed transient bradycardia following DC shock. Complete atrioventricular block lasting for a maximum of 2 hours was the cause in three of these patients. In the remaining

two patients pronounced sinus bradycardia (18-30 beats per minute) was observed in one and persistent atrioventricular dissociation in the other. Digoxin was discontinued for 2-8 days before the treatment in all these five patients.

T wave inversions in the ECG were not recorded in our patients. Systematic enzyme studies were not made in connection with the DC shock therapy. No deaths occurred in direct relation to the DC shock.

2 Of the 437 patients in the present study 13 (3%) experienced an embolic episode in connection with the treatment. All the episodes were observed in relation to successful reversions. Thus the incidence of embolic episodes in the 454 instances of reversion was 2.9%. All the episodes were systemic emboli, seven involved the arteries of the legs, five the cerebral arteries and one the mesenteric artery. Six of the embolic episodes occurred in patients with rheumatic valvular disease and seven in the cases with non-rheumatic etiology of these two were cases of lone atrial fibrillation.

As already mentioned 228 of the patients were on long term anticoagulant therapy during the attempt to achieve reversion of their atrial arrhythmia and 209 were not given anticoagulants. The number of embolic episodes in these groups were two and 11 respectively. This difference is statistically significant. A study is being published separately (2) of the efficacy of anticoagulant therapy in preventing embolism related to DC electrical reversion of atrial arrhythmias.

Pulmonary edema after reversion as observed by Resnekov and McDonald (16) did not develop in any of our patients.

3 The only complication which was believed to be related to the anesthesia was a fall in the systolic blood pressure below 80 mm Hg which occurred in 1 patient. The hypotension was successfully treated with metaraminol and reversion was then carried out without further difficulties.

4 Our policy regarding digitalis dosage in connection with DC shock has already been mentioned. The importance of digitalis as a contributing cause of the disturbances in rhythm observed after DC shock is difficult to evaluate. During the first nine months of the trial we were impressed by the high incidence of ventricular premature

beats and bigeminal rhythm after reversion to sinus rhythm. Consequently in the patients subsequently treated digitalis was discontinued 3-5 days before DC shock was given. We believe that digitalis overdosage was a contributing cause to the development of ventricular tachycardia or ventricular fibrillation in six of our patients.

During quinidine treatment before and after attempts to obtain electrical reversion syncope was observed in eight of the first 230 patients in this series. In four of these syncope occurred before reversion showing that DC countershock was not a contributing cause. Five of the patients had only one attack of syncope but in three instances numerous attacks (15-50) were observed during 5-16 hours ECG monitoring in these patients showed that all the attacks of syncope were due to ventricular fibrillation. This serious complication was heralded in the ECG by marked prolongation of the Q-T interval, ventricular premature beats, bigeminy and short runs of ventricular tachycardia. All the attacks of syncope occurred within three days after quinidine therapy had been started. In spite of the moderate doses of quinidine given (1.2 g/day) the determination of the serum quinidine concentration carried out in four of the patients showed relatively high values (6.3, 6.8, 8.0, 14.7 mg/l) when the attacks were observed. Further details concerning these complications have been published previously (3). A more individual dosage of quinidine, guided by the serum level of quinidine during the treatment has been found to be valuable and new attacks of quinidine syncope have not been observed in the last 117 patients treated with quinidine in this series.

It should also be mentioned in this connection that the five patients who died suddenly between 12 hours and 16 days after reversion with DC shock were all being treated with maintenance doses of quinidine. Therefore the possibility that quinidine may have been a contributing cause can not be excluded in these cases.

As already mentioned 73 patients in this series were treated with a β -blocking agent (H 56/28) in connection with the attempts to obtain electrical reversion of their atrial arrhythmias. No serious complications were observed which could be related to this treatment. The results of this therapeutic trial will be published separately (1).

DISCUSSION AND CONCLUSION

The purpose of a therapy is to benefit the patient. Reversion of an atrial arrhythmia to sinus rhythm is theoretically considered to improve cardiac function, counteract the tendency to congestive heart failure and reduce the risk of systemic and pulmonary embolism. This has been verified or supported by hemodynamic studies (10) and clinical observations.

Assessment of the benefit to the individual patient would have to be based on a detailed and objective clinical and sociological evaluation of the patient's functional capacity during periods of atrial arrhythmia and after sinus rhythm had been well established. We have not embarked upon this endeavour. In a number of our patients it would have been difficult or even impossible to separate the effect of reversion from the effect of other therapeutic procedures, particularly cardiac surgical corrections.

Many patients did however describe considerable improvement and these statements were accentuated by the fact that they felt a definite deterioration when relapse occurred and requested that another attempt be made to obtain reversion. On the other hand a number of patients did not feel any difference between being in atrial fibrillation and being in sinus rhythm and did not recognize the occurrence of a relapse of their arrhythmia.

From the results obtained in this series of patients it is evident that strict criteria cannot be established for the selection of patients who will benefit from reversion. Some trends however seem to be clear and may be of help in the evaluation of the indications.

In relation to etiology (Table III) our best results were obtained in the groups of patients with operated mitral stenosis, operated atrial septal defect and treated thyrotoxicosis. However good long term results in individual patients were observed in all etiological categories and anatomical types of heart disease. No definite difference was found between the total group of rheumatic cases and that of non-rheumatic cases.

The size of the heart (Table IV) had little influence on the results except when excessive enlargement was present. The functional classification of the patients (Table V) had also a very limited predictive value. Much more striking was the relation of the results to the duration of the

preceding arrhythmia (Table VI). The stability of the sinus rhythm attained was found to gradually decrease with increasing duration of the preceding arrhythmia. In patients who had had arrhythmia for more than two years the long term results were rather discouraging and where arrhythmia had been present for more than five years they were poor.

In the evaluation of a therapy the incidence of complications is of decisive importance. The observations made on this series of patients clearly show that the DC shock itself provokes surprisingly few serious complications. This can be emphasized even more strongly when the serious cardiac condition of many of the patients treated is taken into consideration. In spite of this no deaths considered due to the DC shock itself were observed in this relatively large number of patients. In a few cases ventricular arrhythmias including ventricular fibrillation were observed but only one of these proved to be a really dramatic episode. Most of the ventricular arrhythmias occurred in the first period of our trial and some of them were probably provoked because the warnings represented by premature beats before a shock was delivered were not fully taken into account.

Two types of serious complications not directly related to DC shock were observed in the present series, namely systemic embolism and quinidine induced ventricular fibrillation. The embolic complications can probably be appreciably reduced when prophylactic anticoagulant therapy is given. (2) The occurrence of quinidine induced complications was substantially reduced in the last part of this series when more individual dosage of quinidine was used guided by the serum quinidine level. We wish to point out however that the definite value of quinidine in the prophylaxis against relapses of atrial fibrillation is not clearly established as no conclusive controlled clinical trials have ever been carried out.

In conclusion we wish to emphasize that simple criteria for the indications for DC shock reversion of atrial arrhythmias can hardly be given. When not only the DC shock itself but also the anaesthesia and the different types of drugs involved in this treatment are taken into account it amounts to a considerable therapeutic manoeuvre. The indications should be carefully evaluated in each individual patient and the benefits which may

eventually be achieved weighed against the inconveniences, expenses and risks. If all these factors are taken into consideration before the treatment is given we believe that it will remain a valuable part of cardiological therapy.

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RAPID SEMIQUANTITATIVE DETERMINATION OF CHOLINESTERASE ACTIVITY IN SERUM

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Abstract The Acholest test, a simple semiquantitative method for determining plasma cholinesterase, has been investigated. The results of this method agreed well with those obtained by the Warburg manometric method. The test is therefore suitable as a screening test and can be recommended for use prior to regular control of persons exposed to cholinesterase inhibitors, mainly on organophosphorus insecticide exposure.

In recent years the determination of cholinesterase activity in plasma (butyrylcholinesterase, BuChE) and erythrocytes (acetylcholinesterase, AChE) has become of great importance in the appraisal and treatment of poisoning by carbamates and organophosphorus insecticides. Epidemiological studies of persons working with these insecticides have shown that many of them have a reduced cholinesterase activity without showing any clinical symptoms (8).

In 1959-1960 293 samples were taken for the determination of BuChE activity by the Warburg manometric method from 126 workers exposed to organophosphorus insecticides in an agricultural area in South Sweden (19). A good many of the 126 had reduced BuChE activity: 56% had values less than 1.8 U ml^{-1} , 28% less than 1.35 U ml^{-1} and 11% less than 0.3 U ml^{-1} . Of the 54 workers from whom more than one sample was taken during the spraying, 18 (33%) had a reduction of BuChE activity of more than 50% of the highest analysed individual value. Because the sample was taken during the exposure time the normal individual value was probably not obtained for everybody; therefore the reduction of the BuChE activity of more than 50% in one third of the spraymen was rather too low.

The epidemiological investigations and the annual occupational poisoning by preparations of the mentioned type emphasize the need for regular

control with determination of cholinesterase activity of persons exposed to insecticides with cholinesterase inhibiting properties. A control of this nature is arranged in some places (3-6).

The various quantitative methods for determination of cholinesterase activity among which the Warburg manometric method (2) has been predominant in Sweden is hardly suitable for routine control of a large number of exposed persons. Therefore a simple rapid semiquantitative method for determination of cholinesterase activity safe enough for practical health control has been proposed as an obvious need.

Determination of cholinesterase activity is valuable not only on poisoning by cholinesterase inhibitors in agriculture and horticulture but also on exposure to nerve gases. At control of anti-cholinesterase therapy for patients with myasthenia gravis, glaucoma (14) and cancer (20) in the use of succinylcholine chloride for patients undergoing general anaesthesia (18-20) and for diagnosis of liver and kidney disorders (5) it is of practical importance on these occasions to have a rapid simple method that can be managed by untrained personnel and with limited laboratory resources.

Semiquantitative methods for determination of cholinesterase activity are available commercially. The Acholest test (Acholest paper obtainable from Österreichische Stickstoffwerke AG, Linz, Austria) is a simple colorimetric method (17) that determines the BuChE activity in plasma or serum. We have tested Acholest and compared the results with those from the determination of cholinesterase activity by the Warburg manometric method.

METHOD AND TECHNIQUE

Samples were taken from workers with suspected exposure to organophosphorus insecticides, from hospital patients

Table I Number and category of subjects number of samples and Acholest test findings

	No samples	No persons	No persons with Acholest	
			Norm	Decc
Occup insecticide exposure				
in agriculture	11	10	17	3
in horticulture	9	8	7	1
in airplane spraying	20	15	14	1
in manufacturing	12	11	11	
Accidental insecticide exposure	4	4	4	
Anticholinesterase treatment of				
myasthenia gravis	5	3	3	
glaucoma	7	1	1	
Hospital patients with				
cirrhosis of the liver	12	10	2	8
moderate exp. of CCl ₄	2	2	2	
severe lead poisoning	1	2	1	1
nephrosis	3	3	3	
renal insufficiency	10	5	3	2
tetanus (treatment with saxamenton)	1	1	1	
Healthy adults without insecticide exposure	20	15	14	3
Total	135	100	81	19

with various clinical conditions, and from healthy adults not exposed to cholinesterase inhibitors (Table I). At each sampling venous blood and capillary blood were taken at the same time. The venous blood was put directly into tubes and centrifuged after cooling. 0.05 ml capillary blood from the finger tip in a heparinized pipette was applied to filter paper and air-dried. Four such samples were taken. If these were not analysed on the same day the filter papers were kept in a refrigerator.

Acholest Test

Determination with indicator strip was carried out according to the instructions supplied. The analysis was done at room temperature. No temperature correction (16) was made.

Principle

Serum cholinesterase catalyses the following reaction: acetylcholine + H₂O → choline + acetic acid. The test strip is impregnated with a cholinesterase and an indicator (acid = yellow, neutral = blue). On the addition of serum the colour is blue. The acetic acid that originates on acetylcholine decomposition reduces the pH which results in a change of colour from blue-green through green-yellow-green to yellow. The time interval between the serum contact with the test strip and the appearance of the yellow colour with the accompanying control strip is the measure of the cholinesterase activity.

Technique

Two drops of serum, each of 0.05 ml, from the centrifuged venous blood are placed on a well cleaned object glass. With a small forceps half an Acholest strip is placed in one drop and the control strip in the other. A stopwatch is started when the Acholest strip is placed

in the serum and stopped when the colour on this strip coincides with the colour on the control strip. The measured time is the result of the determination. The normal BuChE activity is 6-18 min. 19-35 min is decreased activity and more than 35 min strongly decreased activity.

Warburg Technique

Whole blood is collected from a punctured finger with a heparinized glass pipette. The first drop of the finger tip blood is wiped off with a piece of dry dressing. Exactly 0.05 ml is applied to a filter paper (Munktel, no. 3). Four blood samples are taken for each analysis. The filter papers are air-dried at room temperature. Blood samples on paper are kept in a refrigerator (4°C) until the analysis. The Warburg manometric technique (1, 2, 3) is used to determine the esterase activity in plasma and erythrocytes. The enzyme concentration (esterase activity) is expressed in U/ml, where U is the amount of enzyme that will catalyze the hydrolysis of 1 μ mole of substrate per minute. The normal BuChE activity values for males are 2.20 ± 0.40 and for females 2.03 ± 0.54 U/ml. The normal AChE activity values are 0.95 ± 0.16 and 0.89 ± 0.12 U/ml, respectively (3).

RESULTS

Fig. 1 shows the correlation between BuChE activity determined partly by the Acholest and partly by the Warburg method in 134 samples from 100 persons. There is a good correlation between the two methods. The spread around the mean value 1 and 2 σ (70% and 95%, respectively) for normal Acholest values is ± 0.36 and ± 0.72 U/ml¹.

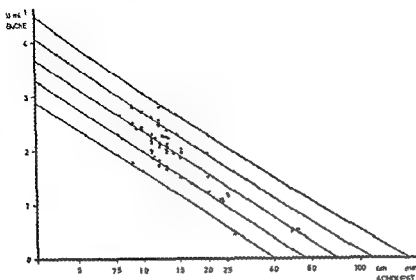


Fig 1 Comparison of the results obtained in 134 blood samples tested for butyrylcholinesterase activity (BuChE) by the Acholtest test and by the Warburg method

respectively. In four samples Acholtest showed reduced activity (18–21 min) whereas the Warburg method would place these values in the normal range. Thirteen samples which lay within normal Acholtest values had decreased activity according to the Warburg method, the lowest being 1.32 U ml⁻¹.

One of the 135 samples as recorded in Table I is not included in Fig 1 because it showed strongly decreased activity. The colour change in the Acholtest strip then occurs so slowly that it is difficult to decide when the colour begins to agree with that of the control strip. Table II shows the results from the person poisoned by an organophosphorus insecticide from whom the omitted sample was taken.

Fig 2 shows the correlation between the BuChE activity determined by Acholtest and AChE activity in erythrocytes determined by the Warburg method. As expected no reliable correlation exists.

To investigate whether Acholtest can be used for determination of BuChE activity in non-fresh serum a daily determination was carried out on the same serum, some of which was kept at room temperature and some in a refrigerator (4°C). Over a period of 10–17 days five samples were investigated (Fig 3). Changes of significance in the Acholtest results appeared in only one sample (no. 1 in Fig 3). In the part of this sample that was kept in the refrigerator the results varied by a maximum of 8 min. In the part kept at room temperature considerable variation occurred with

Acholtest: 30 min on the second day and 13 min on the ninth and eleventh days. This sample kept at room temperature was the only one that showed a tendency to falling cholinesterase activity during this period. In serum from the other four and from those kept up to one week no significant change was found in the Acholtest results during the storage period.

COMMENTS

The investigation has proved the existence of a satisfactory agreement between Acholtest and the Warburg manometric method in the determination of BuChE activity. Similar results were obtained earlier between Acholtest and the mano-

Table II Cholinesterase activity in blood from a 17 year-old male exposed to organophosphorus insecticide, periodically during June and July. 3½ h on 3 Aug and 2 h on 4 Aug. First symptoms observed on 4 Aug. Recovered on 20 Aug.

Warburg method values expressed in U ml⁻¹ i.e. / moles of substrate hydrolyzed per min per mole of whole blood

Date	Acholtest time in min	Warburg method U ml ⁻¹	
		Plasma	RBC
Aug	5	158	0.05
	7	53	0.31
	11	9	0.90
	20	19	1.49
Sept.	9	13	0.07
			0.30

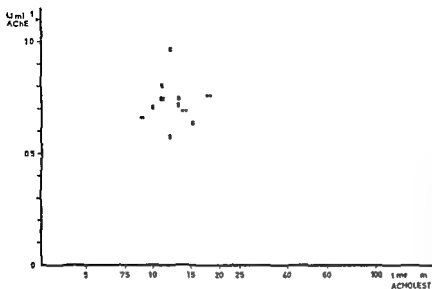


Fig 2 Comparison of the results obtained in 134 blood samples tested for butyrylcholinesterase activity (BuChE) by the Acholest test and for acetylcholinesterase activity (AChE) by the Warburg method

metric method of Ammon (10, 17) and between Acholest and the electrometric method of Michel (8, 16, 20). Although the physiological function of butyrylcholinesterase in plasma is not known, the determination of its activity is of value for forming an idea of the absorption of cholinesterase inhibitor. In connection with the control of workers exposed to insecticides and to nerve gases of the type cholinesterase inhibitor, this test can often

be of greater value than merely to show the degree of the poisoning by determination of the acetylcholinesterase activity. Acholest in this connection is a simple screening test with a satisfactory reliability. Acholest provides the important information as to whether the serum cholinesterase activity is normal or decreased and approximately how much it is decreased. At normal and slightly reduced cholinesterase activity, no difficulties exist in reading when the yellow colour on the Acholest strip agrees with the colour on the control strip. At greater reduction, it may be difficult to determine when the two strips begin to show the same colour, which means that the Acholest value is less precise. This, however, is of less importance. Acholest should be used for practical purposes and not for providing exact values in connection with, for instance, treatment for poisoning or scientific investigations. On these occasions, there are more exact manometric, electrometric, and colorimetric methods (7, 11) which, because of their complicated apparatus and time-demanding procedures, are unsuitable for screening tests.

Acholest does not require laboratory facilities. Persons exposed to cholinesterase inhibitors can be controlled by, e.g., an industrial nurse or district nurse. If normal values are found, the analysis is ready within 30 min. It is a big advantage that a response can be obtained so quickly because the work and the exposure should be discontinued if the cholinesterase activity is low. At Acholest values of more than 30–40 min, the risk for the

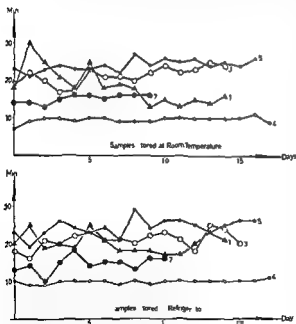


Fig 3 Results of daily Acholest tests on five blood samples stored for a period of 10–17 days at room temperature and in a refrigerator

poisoned person is so great that continued observation should be carried out in a special clinic.

If the analysis cannot be made at once for instance when samples are taken from a large group Acholest gives unchanged values up to at least one week. It is an advantage—although not necessary—that serum be kept in a refrigerator during this period.

Approximately 10% of those in our material who were exposed occupationally had reduced BuChE activity. Our investigation was not an epidemiological study but the persons investigated were from the same area as those investigated by Tejning in 1959–1960 (19). He found reduced BuChE activity in approximately 50% of the spraymen. Comparison of the two investigations indicates that an improvement had occurred: the risk to health had been reduced. During the 1960s an increasing amount of information and training has been provided for workers who handle the toxic pesticides, as from 1964 permission from the authorities is necessary before these substances can be used. However, although the risk of poisoning has decreased quantitatively qualitatively it is still too great because of negligence, accidents, disregard of occupational hygienic measures and unconscious exposure/resorption.

Of the 15 healthy persons who were included in the normal material (Table 1) 12 had normal values with Acholest between 9 and 18 min (2.56–1.58 U ml⁻¹, mean 2.14 U ml⁻¹). Repeated tests from the other three were 19.5 min (1.64 U ml⁻¹), between 20 and 32 min (1.33–1.11 U ml⁻¹) and between 22 and 28 min (1.26–0.9 U ml⁻¹). Liver function tests on all three were normal.

Physiological hypocholinesterasaemia may occur during pregnancy (7). Familial hypocholinesterasaemia (12), familial dyscholinesterasaemia (7, 13), and atypical serum cholinesterase (4) have been described.

Plasma cholinesterase is formed in the liver and moves electrophoretically with α globulin (95%) and with albumin (5%). The production in the liver of plasma cholinesterase and albumin occurs more or less in parallel: a correlation exists between the plasma albumin level and BuChE activity in several diseased conditions (5). Determination of BuChE activity in various hepatic disorders has been reported by several authors (15, 17) and has been judged to be a valuable liver function test, especially in chronic hepatic dis-

orders such as cirrhosis and chronic hepatitis. Of our ten patients with liver cirrhosis eight showed decreased BuChE activity, the lowest being 0.4 U ml⁻¹ (Acholest 98 min).

Acholest under 5 min = understood to mean increased BuChE activity. Hypercholinesterasaemia may occur in adiposis nephrosis and exudative enteropathy (5). In the two latter conditions a compensatory raised albumin synthesis occurs in the liver and parallel with this an increased production of cholinesterase. In our material nobody had hypercholinesterasaemia. Of those investigated 15 had Acholest values less than 10 min and three less than 8 min; there were none less than 6 min. Of these 15 three were patients with the nephrotic syndrome. Acholest was 6 min (3.15), 8 min (2.85) and 8 min (2.07 U ml⁻¹). AChE activity was normal or low: 0.78, 0.75 and 0.57 U ml⁻¹ respectively.

The BuChE activity becomes reduced in connection with dialysis, whereas AChE activity remains unchanged (9). Of our five patients investigated for chronic renal insufficiency three had normal and two slightly increased BuChE activity. The activity was reduced in connection with dialysis in four patients. The reduction was not significant. The AChE activity was low in all five with a mean value of 0.37 (0.23–0.58) U ml⁻¹ but rose significantly in connection with dialysis to 0.60 (0.44–0.82) U ml⁻¹.

CONCLUSION

Those who are exposed to cholinesterase inhibitors in their work are subjected to a serious health risk. Because the cholinesterase activity in the body tissue can be reduced as much as 75% before the first subjective symptoms reveal themselves the risk zone may be very small, i.e. the period from the appearance of the first symptom to the point when a life threatening condition exists. It is therefore of great importance from the occupational hygienic aspect that a regular control of this occupational group be carried out in order to prevent poisoning. Because there now exist simple and rapid semi-quantitative methods for determining the cholinesterase activity in serum such a control ought to take place.

There are hereditary sickness conditions associated with hypocholinesterasaemia; therefore tests for determining the cholinesterase activity

should be made before workers enter employment where there is exposure to cholinesterase inhibitors. For those who spray pesticides this test can be taken in conjunction with the competence course. On seasonal exposure e.g. in agriculture tests can probably also be taken before the beginning of the spraying season. Tests should be taken every week as long as exposure continues. At higher exposure tests are advisable two or three times a week. In the event of occasional strong exposure for instance in connection with spill on skin and clothing or if suspected poisoning symptoms are present tests should be taken on the same day.

Sampling and analysis can be made by a nurse without access to a laboratory. If the worker shows reduced cholinesterase activity (Acholest 20–35 min) he can be transferred to non-exposed work. When the cholinesterase activity returns to normal he can resume his earlier work. At strongly decreased activity (Acholest more than 35 min) a clinic of occupational or internal medicine should take over the responsibility for further measures.

Besides the control of workers who are exposed to insecticides and nerve gases, Acholest and similar simple semiquantitative methods can be of value as control tests in the use of muscle relaxants of succinylcholine type in anaesthesia, cholinesterase inhibitor in myasthenia gravis, glaucoma and cancer, also as diagnostic test in hepatic disorders.

ACKNOWLEDGEMENT

This work was supported by the Swedish Medical Research Council (Project No. 76X 776-01).

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CIRCULATORY STUDIES DURING PHYSICAL EXERCISE IN MENTALLY DISORDERED PATIENTS

1 Effects of Large Doses of Chlorpromazine

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Abstract 1 Nine patients who were not given chlorpromazine (mean age 29 years) and eight patients (mean age 30 years) who received large doses of chlorpromazine (1500-3600 mg/day) were studied with determination of oxygen uptake, ventilation, cardiac output, arterial blood pressure and arterial blood lactate, pyruvate and noradrenaline concentrations at rest, supine and when cycling in the sitting position on a bicycle ergometer with successively increasing work loads. The heart volume was measured roentgenologically with the patient prone. 2 Four patients were studied before and 1.5-5 months after the beginning of chlorpromazine treatment. They maintained their physical performance during that period. 3 All patients had a low physical working capacity on the bicyl ergometer and most of them had a reduced stroke volume in relation to the heart volume. 4 The patients treated with chlorpromazine tended to show further reduction in stroke volume and lower cardiac output during exercise than those who did not receive such treatment. 5 The arterial blood pressure at rest was largely the same in the two groups of patients, but was reduced during exercise in those treated with chlorpromazine. Chlorpromazine did not cause a significant change in peripheral vascular resistance. 6 A high blood noradrenaline level was recorded during exercise after treatment with chlorpromazine. 7 A small stroke volume especially in patients treated with chlorpromazine and the fall in arterial blood pressure in this group are factors that limit physical performance. The importance is stressed of exercise tests in clinical routine in the activation of patients receiving phenothiazines, at least for those receiving large doses.

The physical working capacity of mental hospital patients with chronic disorders is often low (5). Such patients may sometimes be treated with large doses of phenothiazines. Both the physical inactivity and the drug may substantially impair the adaptation of the circulation to physical exercise and thereby retard rehabilitation. It was

therefore thought worthwhile to make a thorough physiological study of a group of patients with chronic mental diseases including some patients receiving large doses of chlorpromazine.

Long term treatment with chlorpromazine results in reduction of the mean arterial blood pressure in upright posture (18) but no studies have reported on the effect of such treatment on the arterial pressure during exercise. Certain unexplained cases of sudden death (9, 13) among patients receiving phenothiazines may have been due partly to maladaptation of the circulation. Carlsson et al (6) reported an increased noradrenaline concentration at rest in the blood plasma and urine in patients receiving large doses of chlorpromazine. The difference in noradrenaline level between such patients and patients not receiving the drug was still more striking during physical exercise.

This investigation which is a continuation of the study just mentioned (6) is concerned with the effects of physical inactivity and of large doses of chlorpromazine on circulatory adaptation to physical exercise. Patients who had been receiving large doses on clinical grounds were selected for the study. Some of the patients took part in a physical training programme reported in the following article in this journal (7).

MATERIAL

The material is summarized in Table I. Group I (9 males) mean age 9 years (range 19-42) consisted of patients who had not received chlorpromazine. Group II (11 males) mean age 30 years (range 23-50) consisted

Table I *Anthropometrical and clinical data*

Pat no	Age (y)	Weight (kg)	Height (cm)	Diagnosis	Duration of disease (y)	Medicine				Duration
						Chlorpromazine			Additional drugs daily dose	
						Dose (mg/day)	Duration (mo)			
Group I										
1	19	57	170	Schizophrenia	3	0	—			
2	37	64	167	Schizophrenia	20	0	—			
3	42	77	167	Schizophrenia	20	0	—	Phenytoin (100 mg × 3)	phenobarbitone (100 mg × 3)	2 mo
4	31	57	176	Schizophrenia	6	0	—	Amylobarbitone 100 mg		Temporarily
5	8	63	176	Schizophrenia	6	0	—			
13	19	62	183	Schizophrenia	1	0	—			
14	33	92	167	Mania	15	0	—			
15	20	60	174	Schizophrenia	1	0	—			
18	30	80	176	Anxiety neurosis	3	0	—			
Group II										
5	28	63	176	Schizophrenia	6	2000	15			
6	31	108	185	Schizophrenia	11	3000	4			
7	43	87	171	Schizophrenia	9	3600	> 12			
8	23	101	181	Schizophrenia	3	1800	1			
9	26	118	179	Schizophrenia	6	2500	4			
10	23	0	175	Schizophrenia	2	1500	> 12	Benztropine (2 mg × 1)	n-ethyl norphenyl ephrine hydrochloride (Effonil®) (5 mg × 3)	2 mo and 1 mo resp
11	27	99	181	Schizophrenia	5	2400	> 12	Benztropine (2 mg × 1)	n-ethyl norphenyl ephrine hydrochloride (Effonil®) (20 mg × 3)	2 mo
12	50	69	174	Schizophrenia	8	2100	> 12	Benztropine (2 mg × 1)	amphetamine (5 mg × 2)	

of those who had received large doses of chlorpromazine for more than one month. Most patients had been ill for several years, some of them for even more than a decade during which they had been physically rather inactive. The patients were informed about the experimental nature of the study and all agreed to cooperate.

Four patients (nos 1, 2, 4 and 5) were studied both before and 15–5 months after the beginning of treatment with chlorpromazine with final doses of 300, 300, 900 and 2000 mg/day respectively. Patients 1, 2 and 4 had before insertion of the drug taken part in the physical training programme for 15–3 months (ref. 7). They continued their physical training after application of chlorpromazine and kept their physical activity fairly

constant. The total number of patients receiving large or rather large doses of phenothiazines in the hospital is considerable. But the frequency of new inpatients requiring such large doses of phenothiazines is very low. It was considered justifiable, however, to include this small group in our report.

METHODS

The patients arrived at the laboratory in the morning. The heart volume was determined roentgenologically with the patient prone (19). Polyethylene catheters were inserted percutaneously (7) into a brachial artery and a

peripheral arm vein. The tip of the venous catheter was placed proximally in the axilla. The plasma volume was then determined with ^{125}I albumin (RISA). The total blood volume was calculated from hematocrit values obtained for peripheral arterial blood corrected for trapped plasma and for body hematocrit.

The cardiac output was determined with the indicator dilution technique using ^{51}Cr Hippuran (α -iodohippuric acid sodium salt, obtained from Radiochemical Centre, Amersham, England, 20–40 μCi in a volume of 10 ml for each injection) as indicator and fraction collector for the arterial samples. The technique has been discussed by Björk (4) who found good agreement between the cardiac output determined with the Hippuran method and with the direct Fick method.

Resting electrocardiograms comprising the standard leads I, II, III, the augmented unipolar extremity leads aVR, aVL, aVF and the precordial leads CR, CR₁, CR₂, CR₃, CR₄ were recorded on a direct writing manganograph (Elema, Sweden). During exercise the indifferent electrode was placed on the forehead (CH leads) and CH₁, CH₂, CH₃ and CH₄ were recorded. The heart rate was counted from the electrocardiogram using not less than 25 beats.

The intraarterial blood pressure was recorded with an inductance manometer (Elema, Stockholm) and the mean pressure was obtained by electrical integration. When the patient was supine a level, 5 cm below the angle of Ludwig was used as reference and when sitting, the connection of the fourth rib to the sternum.

The oxygen uptake was measured by collecting expired air in Douglas bags and analysing it with the micro-Scholander technique.

The hemoglobin concentration in arterial blood was determined spectrophotometrically after conversion to cyan methemoglobin, according to Drabkin and Austin (8). The oxygen saturation in arterial blood was determined spectrophotometrically as described by Holmgren and Pernow (16).

Lactic acid was determined on arterial blood according to Strom's (2') modification of the Baker and Summerson method. In most patients it was also estimated by an enzymatic method (1). Pyruvic acid was determined by an enzymatic spectrophotometric method (10).

The noradrenaline concentration in arterial plasma was estimated spectrophotometrically according to the principles of the indolehydroxyindole reaction after the samples had been passed through a column of strong cation exchange resin (17).

The hemodynamic studies were started at the earliest two hours after the patients had had their regular breakfast. Measurements were first made with the patient resting in the supine position. The patients afterwards sat on the bicycle ergometer (14) and the arterial blood pressure was recorded after about 2–4 min in this position. Exercise was then started and was done with successively increasing work loads with 10 min pauses during which the patient rested in a chair. The BP and ECG were recorded every second min. The cardiac output was determined after exercise for 6 min with each load. The BP was recorded immediately before and after determination of the cardiac output. The expired

air was collected during determination of the cardiac output. After exercise for about 8 min with each work load, or occasionally immediately and 3–4 min after a shorter period of work if the patient found the exercise very strenuous, blood was collected for measurement of lactic acid and pyruvic acid.

After exercise for 9 to 11 min arterial blood was sampled for determination of the noradrenaline concentration.

The highest work load was chosen so that the patient would be exhausted after the exercise period. In patients 5 and 11 the highest work load could only be maintained for a short period and the hemodynamic observation was made already after 3–4 min of exercise. Such results were not used in the calculation of mean values, but are nevertheless included in Tables II and III. All the patients had performed a regular exercise test with recording of the ECG before the examination proper; this was also carefully explained to the patients. No complications, excepting minor hematomas, were observed.

RESULTS

Comparison between groups I and II

The results obtained for patients who had not received chlorpromazine (group I) and for those who had (group II) are given in Tables II and III. Table IV shows the mean values for pertinent data in the two groups.

The highest work load which could be maintained for at least 6 min usually 10–11 min was on the average 739 (range 300–900) kpm/min in group I and 563 (range 300–900) kpm/min in group II.

It was not possible to obtain samples of expired air from all the patients because some of them did not tolerate the mouthpiece or the noseclip. In group I the oxygen uptake at rest was 114 l (range 84–150) of the predicted basal metabolism (12) and in group II it was 106 (range 90–130). No systematic difference in oxygen uptake at rest or during exercise was observed between the two groups. The values during exercise were of the same order as those found in comparable studies of healthy men of similar ages (e.g. 10, 11).

The total ventilation at rest and during light exercise was sometimes higher in group II than in group I.

The average arterial hemoglobin concentration at rest was largely the same in both groups (I = 14.0 and II = 13.7 g/100 ml). The corresponding values noted for the highest work load were 14.7 and 14.6 g/100 ml. The arterial oxygen

Table 11 Results of physiological tests at rest and during exercise in untrained patients without chlorpromazine

Pat. no	Work load	Oxygen uptake STPD (l/min)	Ventilation BTPS (l/min)	Cardiac output (l/min)	Heart rate (beats/min)	Stroke volume (ml)	Blood pressure (mm Hg)			Lactate (mM/l)	Pyruvate (mM/l)	Noradrenaline (μ g/l plasma)
							Syst	Diast	Mean			
1	Rest	0.33	9.6	8.3	90	92	177	66	91	0.88	0.06	0.8
	Supine											
	660 ml Sitting	0.61	12.2	12.2	138	77	155	96	116	2.08	0.14	1.4
	4.3 l 300	1.03	22.9	13.5	187	72	139	91	112	3.33	0.17	2.1
	14.3 g/100 ml 750	1.41	33.6	13.3	207	64	136	105	121	9.18	0.25	2.7
2	Rest	0.26	6.2	7.0	83	85	131	67	87	0.69	0.04	0.7
	Supine											
	600 ml Sitting	0.86	19.0	8.8	106	83	149	88	94	0.78	0.04	1.3
	4.6 l 600	1.43	31.4	10.03	139	74	135	69	95	1.49	0.05	2.6
	13.9 g/100 ml 900	2.06	52.3	12.8	171	72	141	73	100	5.33	0.06	2.9
3	Rest	0.76	10.1	5.9	74	80	132	73	103	1.36	0.06	0.7
	Supine											
	820 ml Sitting	0.83	19.4	9.0	116	78	167	90	116	4.48	0.20	1.3
	3.9 l 300	1.05	30.0	9.2	146	63	175	96	122	6.09	0.23	2.1
	14.7 g/100 ml 500											
4	Rest	0.18	5.4	5.1	69	74	106	56	74	1.08	0.06	0.5
	Supine											
	620 ml Sitting	0.83	22.3	10.4	108	96	161	78	101	3.53	0.17	1.3
	5.0 l 300	1.59	64.9	—	164	—	197	96	137	8.73	0.24	2.6
	10.3 g/100 ml 600											
5	Rest	0.33	10.0	13.3	120	111	144	77	96	0.96	0.06	0.3
	Supine											
	720 ml Sitting	0.95	26.1	14.0	144	97	171	97	129	2.38	0.11	0.6
	5.5 l 300	1.54	45.5	14.9	152	98	163	77	109	4.58	0.15	—
	14.5 g/100 ml 600	2.19	68.9	17.5	178	88	157	81	118	7.24	0.15	4.0
13	Rest	0.22	5.3	4.6	61	74	120	61	81	(0.95)	0.08	
	Supine											
	610 ml Sitting	0.89	17.9	10.3	90	114	131	74	99	—	—	
	4.2 l 300	1.42	30.6	13.5	152	89	179	90	114	(4.33)	0.15	
	14.0 g/100 ml 600											
14	Rest	0.28	7.7	7.3	81	94	131	70	97	1.44	0.10	
	Supine											
	800 ml Sitting	0.90	21.3	—	116	—	155	90	109	—	—	
	15.1 g/100 ml 300	1.01	26.1	—	124	—	169	86	113	2.01	0.13	
	15.1 g/100 ml (600)						173	81	99	2.04	0.14	
15	Rest	0.78	6.9	7.3	77	95	166	91	116	(0.97)	0.07	
	Supine											
	4.1 l Sitting	1.06	23.4	14.1	113	125	156	91	115	—	—	
	13.1 g/100 ml 300	1.49	41.2	18.3	143	128	186	104	132	(1.99)	0.13	
	13.1 g/100 ml 600			21.1	175	121	219	103	143	(4.49)	0.23	
18	Rest	0.28	7.3	7.9	84	94	125	65	92	0.73	0.07	
	Supine											
	920 ml Sitting	0.99	24.8	10.3	117	88	187	85	119	0.97	0.07	
	14.5 g/100 ml 300	1.30	34.0	12.3	141	87	205	85	117	1.67	0.12	
	14.5 g/100 ml 600	1.98	48.4	15.8	172	92	213	80	113	3.38	0.13	

Left column gives subject's number heart volume blood volume and hemoglobin concentration at rest

Table III Results of physiological tests at rest and during exercise in untrained patients receiving large doses of chlorpromazine

Pat. no	Work load	Oxygen uptake STPD (l/min)	Ventilation BTSP (l/min)	Cardiac output (l/min)	Heart rate (beats/min)	Stroke volume (ml)	Blood pressure (mm Hg)			Lactate (mM/l)	Pyruvate (mM/l)	Noradrenaline (μ g/l plasma)
							Syst.	Diast.	Mean			
5	Rest	0.30	9.4	6.8	92	74	115	65	84	(0.38)	0.10	1.1
—	Supine											
—	Sitting											
—	300											
13.1 g/100 ml	600	0.88	23.1	8.9	130	68	136	67	97	(1.30)	0.08	1.1
—	900	1.55	41.1	11.1	160	70	157	74	107	(4.33)	0.14	5.5
—	1200	2.13	66.7	14.5	175	81	162	78	120	(6.38)	0.21	7.0
6	Rest	—	—	6.2	81	76	117	75	95	0.71	0.04	—
—	Supine											
—	Sitting											
—	300											
850 ml	600	—	—	10.0	120	83	132	80	99	2.03	0.07	—
6.6 l	900	—	—	12.2	131	93	140	75	99	3.77	0.08	—
12.5 g/100 ml	1200	—	—	—	—	—	—	—	—	—	—	—
7	Rest	—	—	7.1	112	81	130	80	98	0.70	0.07	2.0
—	Supine											
—	Sitting											
—	300											
740 ml	600	—	—	10.2	137	74	166	87	115	1.95	0.14	5.4
4.8 l	900	—	—	10.5	150	70	177	88	118	3.64	0.16	15.0
13.2 g/100 ml	1200	—	—	—	—	—	—	—	—	—	—	—
8	Rest	0.28	7.8	7.7	100	77	131	78	98	0.95	0.05	0.8
—	Supine											
—	Sitting											
—	300											
620 ml	600	0.91	24.9	10.3	132	78	165	79	101	1.86	0.11	3.9
—	900	1.58	51.0	14.5	150	97	143	63	88	3.77	0.14	5.9
14.6 g/100 ml	1200	—	—	—	—	—	—	—	—	—	—	—
9	Rest	0.25	8.7	10.9	120	91	134	71	92	0.78	0.05	0.9
—	Supine											
—	Sitting											
—	300											
520 ml	600	0.89	27.3	10.0	136	74	158	91	108	1.11	0.11	4.1
4.8 l	900	1.47	48.4	11.4	152	75	135	60	86	4.33	0.09	4.5
13.4 g/100 ml	1200	—	—	—	—	—	—	—	—	—	—	—
10	Rest	0.24	8.4	6.1	100	61	128	74	94	0.58	0.05	0.9
—	Supine											
—	Sitting											
—	300											
500 ml	600	0.92	29.7	10.0	144	70	176	83	112	2.89	0.14	1.5
3.3 l	900	—	—	—	—	—	—	—	—	—	—	—
14.2 g/100 ml	1200	—	—	—	—	—	—	—	—	—	—	—
11	Rest	0.29	7.7	8.3	98	85	137	85	106	2.10	—	1.5
—	Supine											
—	Sitting											
—	300											
750 ml	600	—	—	10.6	137	77	145	96	127	1.69	—	4.7
7.6 l	900	—	—	11.2	158	71	159	76	101	3.50	—	2.7
14.4 g/100 ml	1200	—	—	—	—	—	—	—	—	—	—	—
12	Rest	0.25	9.8	5.3	87	61	145	83	109	1.14	0.09	1.0
—	Supine											
—	Sitting											
—	300											
860 ml	600	—	—	6.5	106	62	139	87	108	3.16	0.15	5.2
4.3 l	900	—	—	—	—	—	—	—	—	—	—	—
13.9 g/100 ml	1200	—	—	—	—	—	—	—	—	—	—	—

Left column gives subject's number, heart volume, blood volume and hemoglobin concentration at rest.

saturation was normal both at rest and during exercise. The mean resting values for groups I and II were 97.8 and 97.7%. The values for the highest work load were 97.6 and 98.2 respectively.

Fig. 1 shows the cardiac output in relation to

the oxygen uptake (or work load). The cardiac output varied widely in three subjects not treated with chlorpromazine (nos. 1, 5 and 15); it was large during exercise whereas in some patients in group II (especially no. 12) it was relatively small. The mean values in group I were of the

Table IV Mean values for the two groups I without II with chlorpromazine

		Exercise—Sitting									
		Rest		supine		(300 kpm/min)		(600 kpm/min)		(900 kpm/min)	
		I	II	I	II	I	II	I	II	I	II
Oxygen uptake	n	9	6	9	4	6	3	3	3	---	---
STPD (l/min)	Mean	0.27	0.22	0.88	0.90	1.50	1.53	2.06	---	---	---
Ventilation	n	9	6	9	4	6	3	3	3	---	---
BTSP (l/min)	Mean	7.6	8.6	20.7	26.3	41.3	46.8	56.5	---	---	---
\dot{V}_E/\dot{V}_O	n	9	6	9	4	6	3	3	3	---	---
	Mean	28.5	32.5	23.4	29.2	27.3	30.6	27.1	---	---	---
Cardiac output	n	9	8	8	8	5	5	4	---	---	---
(l/min)	Mean	7.4	7.3	11.1	9.6	13.9	13.9	16.8	---	---	---
Heart rate	n	9	8	9	8	8	6	4	---	---	---
(beats/min)	Mean	82	99	119	130	148	150	174	---	---	---
Stroke volume	n	9	8	8	8	5	5	4	---	---	---
(ml)	Mean	89	76	95	73	95	81	96	---	---	---
Arterio-venous oxygen difference (ml/l)	n	9	5	8	3	5	---	3	---	---	---
	Mean	38	36	81	90	108	119	137	---	---	---
Brachial artery pressure (mm/Hg) systolic	n	9	8	9	8	6	6	4	---	---	---
	Mean	131	130	166	147	187	152	183	---	---	---
Brachial artery pressure (mm/Hg) diastolic	n	9	8	9	8	6	6	4	---	---	---
	Mean	70	76	87	78	87	73	85	---	---	---
Brachial artery pressure (mm Hg)	n	9	8	9	8	6	6	4	---	---	---
	Mean	93	97	115	101	118	100	120	---	---	---
TPVR ($\frac{\text{mm Hg}}{\text{l/min}}$)	n	9	8	8	8	5	5	4	---	---	---
	Mean	13.5	13.8	10.5	10.7	8.5	8.5	7.2	---	---	---
Lactate (mM/l)	n	7	7	7	7	4	5	3	---	---	---
	Mean	1.1	1.0	2.3	2.2	4.2	4.2	5.3	---	---	---
Pyruvate (mM/l)	n	9	7	8	7	6	5	4	---	---	---
	Mean	0.07	0.07	0.12	0.11	0.16	0.12	0.14	---	---	---
Noradrenaline ($\mu\text{g/l}$ plasma)	n	5	7	5	7	2	4	2	---	---	---
	Mean	0.60	1.17	1.18	3.70	2.60	7.73	3.45	---	---	---

same order in rest and during exercise at 600 and 900 kpm/min as those found for sedentary men aged 21–39 by Grimby et al (11) where the corresponding values were 6.9, 13.6 and 16.7 l/min. The mean values during exercise were lower in group II than in group I.

The resting heart rate (Fig. 2) in both the supine and the sitting position tended to be higher in group II (99 and 108 beats/min) than in group I (82 and 87 beats/min). This tendency was also seen in some patients during exercise at 300 kpm/min but not at heavier work loads. One patient (no. 1 in group I) showed a special pattern with very high heart rate even during light exercise and a low arteriovenous oxygen difference in relation to the heart rate.

The stroke volume tended to be smaller in group II than in group I (Fig. 3). The maximal stroke volume during exercise was related to the heart volume measured roentgenologically in the prone position (Fig. 4). In most patients the

stroke volume was small in relation to the heart volume and in 11 of 14 patients it was below the regression line observed by Astrand et al (1) for young healthy men. It was also noteworthy (Fig. 3) that in five of eight patients in group II the largest stroke volume was recorded during exercise with the largest load but never in any of the patients in group I.

The average arterial systolic pressure when the patient was resting in the supine position was largely the same in both groups. In both groups it increased when the patients sat up; it was then 144 mm Hg in group I and 148 mm Hg in group II. During exercise however it tended to be lower in group II than in group I. In four of eight patients in group II it was even lower when they were exercising at 300 kpm/min than when they were sitting at rest on the bicycle. This blood pressure pattern was not seen in group I or in a control material of healthy men of the same ages (10). In Fig. 5 the systolic

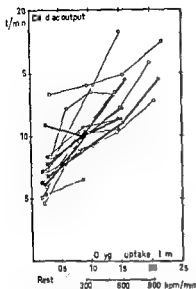


Fig 1 Cardiac output in relation to oxygen uptake (work load) at rest supine and during exercise in sitting position Group I (without chlorpromazine) unfilled rectangles at rest unfilled circles during exercise group II (with chlorpromazine) filled rectangles at rest, filled circles during exercise

blood pressure is related to the heart rate. Practically all values in group II fell below the dotted line connecting the mean values found in a control material (10). The diastolic blood pressure



Fig 2 Heart rate in relation to oxygen uptake (work load) at rest supine and during exercise in sitting position. For explanation of symbols see Fig 1

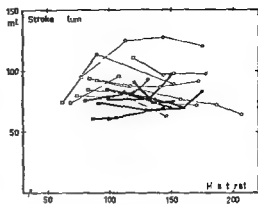


Fig 3 Stroke volume in relation to heart rate at rest supine and during exercise in sitting position. For explanation of symbols see Fig 1

sure tended to show a similar difference between the two groups. When the patients who were sitting at rest began to exercise at 300 kpm/min the pressure decreased in all of them (except one) in group II but only in three out of nine in group I. At rest the mean arterial pressure was equal in both groups but during exercise it tended to be lower in group II than in group I. In six of eight patients in group II the pressure was lower when they were exercising at 300 kpm/min than when sitting at rest. In two of six patients it fell still further when the work load was increased to 600 kpm/min. In three patients in group I a similar tendency was noted at the heaviest work load but not at 300 kpm/min.

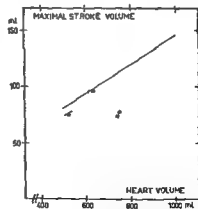


Fig 4 Maximal stroke volume during exercise in relation to heart volume measured in prone position. For explanation of symbols see Fig 1. The straight line with out symbols gives the relationship in healthy young men reported by Astrand et al. (1) the broken lines give the standard deviation

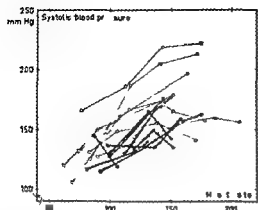


Fig 5 Brachial artery systolic blood pressure in relation to heart rate at rest supine and during sitting exercise. For explanation of symbols see Fig 1. The dotted line without symbols connects values in a group of healthy men (aged 23-39 years) reported by Grimby (10).

In one subject (no 12 in group II) the arterial blood pressure fell markedly during light exercise. The stroke volume was small both at rest and during exercise and during exercise the heart rate was relatively low resulting in a small cardiac output during exercise at 300 lpm/min (Fig 1). The lactic acid concentration increased considerably indicating that the oxygen supply to the working muscles was insufficient. For this work load the noradrenaline concentration also showed one of the most substantial increases. In this patient the blocking effect of chlorpromazine had presumably been unusually strong. He was originally selected for physical training but his poor physical performance and the results of the physiological studies induced us not to accept him for this purpose.

The total peripheral vascular resistance was

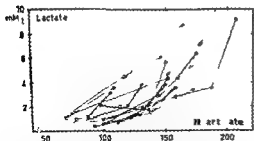


Fig 6 Arterial lactate concentration in relation to heart rate at rest supine and during exercise in sitting position. For explanation of symbols see Fig 1. The dotted line connects mean values in a group of healthy men (aged 23-39 years) reported by Grimby (10).

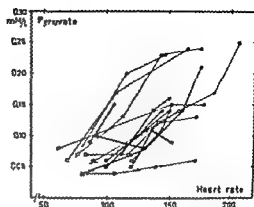


Fig 7 Arterial pyruvate concentration in relation to heart rate at rest supine and during exercise in sitting position. For explanation of symbols see Fig 1. The dotted line connects mean values in a group of healthy men (aged 23-39 years) reported by Grimby (10).

calculated and the average values for the two groups were practically identical (Table IV). The fall in mean arterial pressure therefore seems to have been due to a relatively small cardiac output.

The blood lactate concentration is plotted against the heart rate in Fig 6. In most of the patients in group II the marked increase occurred at a lower heart rate than in a control material (10) or in most subjects in group I. Two of the patients in group I (nos 3 and 4) had however high values both at rest and during exercise. These two patients together with two others in group I (nos 14 and 15) and one (no 12) in group II had also high values for pyruvate whereas in most of the other patients these values were roughly the same as those in Grimby's (10) control material (Fig 7).

The electrocardiogram was normal in all patients in group I before, during and after exercise. In recordings at rest the T waves were flattened in seven of nine patients in group II usually in association with tachycardia. No abnormal ECG findings were noted during exercise. In three of the patients the flattening of the T waves was less marked four minutes after than before exercise. No arrhythmia was recorded.

Comparison of data before and after commencement of treatment with chlorpromazine. In four patients comparisons could be made before and after treatment with chlorpromazine had

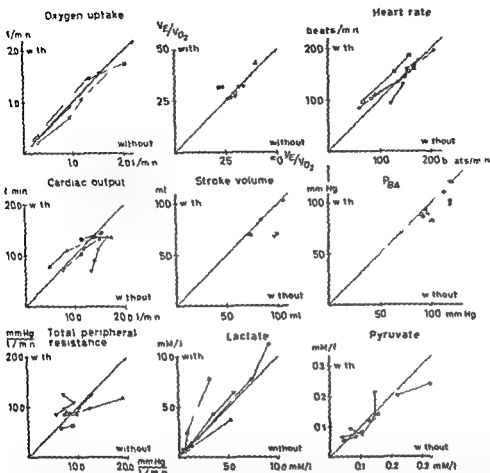


Fig 8 Oxygen uptake ventilatory equivalent, heart rate, cardiac output, stroke volume, brachial artery mean pressure, total peripheral resistance, lactate and pyruvate concentration without and with chlorpromazine in four

patients. The straight line is the identity line. Unfilled symbols at rest supine; filled symbols during exercise in sitting position. Patient no 1 (○), no 2 (□), no 4 (△), no 5 (▽).

been started (Fig 8). Three of these four patients (nos 1, 2 and 4) had taken part in the physical training programme (7) for 1.5–3 months immediately before treatment with the drug. They continued their training and maintained their physical performance at a fairly constant level after chlorpromazine treatment had started. According to the results in the following paper (7) the physical training may if anything have tended to suppress some of the most important circulatory effects of chlorpromazine, namely the high noradrenaline level in blood and the low stroke volume.

The oxygen uptake did not show any systematic change after treatment with chlorpromazine had been started. In three of the four subjects the ventilatory equivalent (\dot{V}_E/\dot{V}_{O_2}) was higher during

exercise after than before administration of chlorpromazine. In three of the four subjects the heart rate at rest was higher after than before treatment with chlorpromazine had been started. During exercise the differences tended to disappear. The cardiac output did not show any systematic tendency—except lower values at the heaviest work load—after treatment with chlorpromazine had been started. During exercise all corresponding values of the stroke volume, with one exception (no 4 at 300 kpm/min), were lower after than before chlorpromazine had been given. This agrees well with the findings when comparing groups I and II. All corresponding values for mean arterial blood pressure, with two exceptions (no 4 at 300 kpm/min and no 5 at 900 kpm/min), were lower after than before

chlorpromazine administration *Total peripheral vascular resistance* however showed fairly wide individual variation but no consistent tendency. In three of the four patients when exercising the *lactate concentration* increased more during than before treatment with chlorpromazine. The *pyruvate* values did not show any clearcut changes after chlorpromazine therapy had been started.

DISCUSSION

The investigation of the circulation at rest and during exercise indicates that large doses of chlorpromazine reduce the arterial blood pressure and the stroke volume which sometimes affects physical performance. The drug had no demonstrable effect on the total peripheral vascular resistance. Hence the fall in mean arterial pressure can probably be ascribed to the small cardiac output. This in turn is due to reduction of the stroke volume without a sufficient compensatory increase in the heart rate. Small cardiac output and low blood pressure are sometimes seen even during light exercise but at other times occur only during strenuous exercise.

The effects of chlorpromazine on the cardiovascular system are complex. The drug has not only a direct influence on the heart and blood vessels but also influences them indirectly e.g. by its action on the central nervous system and autonomic reflexes. Hypotension after initial administration may primarily be due to inhibition of centrally mediated pressor reflexes but peripheral α -adrenergic blockade may also play a role.

The reduction of the stroke volume during light exercise may be due to a decreased vasoconstrictor tone of the capacitance vessels caused by the central and peripheral effects of the drug. The normal reaction to exercise is an increase in venomotor tone (3).

The vasoconstrictor tone in the resistance vessels tends at most to be slightly influenced by the drug. The primary effect of the drug may perhaps be vasodilatation followed by a compensatorily increased sympathetic activity as an adaptation to the repeated large doses of the drug. This may explain the increased noradrenaline outflow but other mechanisms may also be of importance. Some of them have been discussed in an earlier paper (6). The increased sympathetic activity however may be insufficient to main-

tain the stroke volume by normalizing the tone of the capacitance vessels.

The effects of adrenergic blocking agents on metabolic processes in man appear to be complex. The effects on carbohydrate metabolism in the liver do not readily fall into α or β types of activity but with respect to muscle glycogenolysis and lacticidemic action it seems to be mediated via β receptors (for discussion see ref 20-22).

Noteworthy in this connection are the results of the comparison before and after treatment with the drug which showed that after chlorpromazine treatment the patients—who had higher blood levels of noradrenaline and a receptor blockade to some degree—in three cases out of four had higher blood lactate levels during exercise. The results suggest that further metabolic studies might be of interest.

It may also be questioned whether the high noradrenaline levels in blood do not imply a particular risk for the patients owing to some effect on the β receptors in the heart especially with reference to the sudden death of patients who received large doses of phenothiazines (9-13). In the present study however no remarkable ECG findings were noted except flattening of the T waves.

The physical performance must be considered low in both the treated and untreated patients. The low stroke volume seems to be one of the factors limiting the physical performance in both groups. Both the phenothiazine treatment as discussed above and the long period of physical inactivity can be the causes of the low stroke volume. Some patients in group I (nos 1, 5 and 15) had a hyperkinetic circulation during exercise with a relatively low arteriovenous oxygen difference even during exercise with the heaviest load. This is probably caused by an inadequate regulation of the peripheral blood flow during exercise. A similar circulatory dysfunction during exercise is seen in the syndrome vasoregulatory asthenia described by Holmgren et al (15).

The fall in arterial blood pressure during exercise in most patients in group II reduced the tolerance to physical exercise and rendered even the training programme impossible for one of the patients. However a fall in blood pressure at rest in the standing position as in the study by Korol et al (18) was not observed in our pa-

tients when sitting on a bicycle. It should be noted that our patients were well adapted to large doses. The fall in blood pressure during exercise indicated that work tests including registration of blood pressure are useful in clinical routine in activation of patients receiving large doses of chlorpromazine. Whether similar effects of any significance occur also after treatment with more moderate doses is not yet known. A pronounced sudden fall in blood pressure during exertion together with the high noradrenaline level might have been the mechanism involved in the cases of sudden death described by Greiner et al (9) and Hollister et al (13).

ACKNOWLEDGEMENTS

This work has been supported by grants from the Swedish State Med. Cal. Research Council (nos F 0166B A and 14X 166-0, and -03) the AB Leo Fund for Research and the Folksam Research Fund for Rehabilitation.

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CIRCULATORY STUDIES DURING PHYSICAL EXERCISE IN MENTALLY DISORDERED PATIENTS

II Effects of Physical Training in Patients with and without Administration of Chlorpromazine

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Abstract 1 Circulatory studies were performed on four schizophrenics (mean age 27 years) not treated with chlorpromazine and on five (mean age 30 years) treated with large doses of this drug (1800-3600 mg/day) before and after 15-4 months of physical training. 2. The physical training consisted of strenuous exercise on a bicycle ergometer for three 6-minute periods five times a week. 3. After training, all the patients except two could cycle with a larger work load for 6-10 minutes. 4. In the drug free patients the heart rate and arterial blood lactate decreased with training. This training effect was less consistent in the patients treated with chlorpromazine. 5. The stroke volume was small in relation to the heart volume before training. It tended to become normal in five of eight patients after training. 6. In the patients treated with chlorpromazine the blood noradrenaline level was high during exercise and fell in all the patients except one after training. In the drug free patients this level was not as high and there was a further decrease with training in all four patients.

In the preceding paper (4) the physical performance of a group of patients with chronic mental disorders was reported as low. Physical inactivity was assumed to be one of the main causes of this weakness. Some of the patients were receiving large doses of chlorpromazine which might have contributed to their poor physical performance. In an earlier investigation we found that even patients with severe mental disease could take part in a physical training programme with good results (2).

This paper is concerned with the effect of a short period of physical training on the circulation during exercise. In normal men such training has been shown to increase the stroke volume (5-10) and the arteriovenous oxygen difference

(5-10) resulting in an increased maximal cardiac output and aerobic power (maximal oxygen uptake). In patients with coronary disease increased arteriovenous oxygen difference and decreased cardiac work was observed at submaximal work loads after training (11). It was of special interest to study the effects of training on the patients receiving large doses of chlorpromazine. According to a previous study (3) training tended to lower the high blood level of noradrenaline in these patients.

The present series consists of only a few patients. This is because as a rule patients of this type cannot manage without drugs for several months. In the group given drugs only those patients were included who had been treated almost exclusively with one drug (chlorpromazine) in a large dose. This also limited the number of patients available. Despite this selection such patients form a rather heterogeneous group. Our results should therefore be regarded partly as case reports of patients taking part in a physical training programme.

MATERIAL

Four male schizophrenics not treated with chlorpromazine (nos 1, 2, 4 and 13 in reference 4)—mean age 27 years—and five male patients treated with the drug (1800-3600 mg/day nos 6, 7, 8, 9 and 11 in reference 4)—mean age 30 years—took part in the physical training programme. They were studied before and after 3-4 months of training, except for nos 1, 4 and 13 who had to be studied already after 1-3 months training, because they could no longer manage without chlorpromazine.

CIRCULATORY STUDIES DURING PHYSICAL EXERCISE IN MENTALLY DISORDERED PATIENTS

II Effects of Physical Training in Patients with and without Administration of Chlorpromazine

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Abstract 1 Circulatory studies were performed on four schizophrenics (mean age 27 years) not treated with chlorpromazine and on five (mean age 30 years) treated with large doses of this drug (1800-3600 mg/day) before and after 1.5-4 months of physical training. 2. The physical training consisted of strenuous exercise on a bicycle ergometer for three 6-minute periods five times a week. 3. After training, all the patients, except two, could cycle with a larger work load for 6-10 minutes. 4. In the drug-free patients the heart rate and arterial blood lactate decreased with training. This training effect was less consistent in the patients treated with chlorpromazine. 5. The stroke volume was small in relation to the heart volume before training. It tended to become normal in five of eight patients after training. 6. In the patients treated with chlorpromazine the blood noradrenaline level was high during exercise and fell in all the patients except one after training. In the drug-free patients this level was not as high, and there was a further decrease with training in all four patients.

In the preceding paper (4) the physical performance of a group of patients with chronic mental disorders was reported as low. Physical inactivity was assumed to be one of the main causes of this weakness. Some of the patients were receiving large doses of chlorpromazine which might have contributed to their poor physical performance. In an earlier investigation we found that even patients with severe mental disease could take part in a physical training programme with good results (2).

This paper is concerned with the effect of a short period of physical training on the circulation during exercise. In normal men such training has been shown to increase the stroke volume (5, 6, 10) and the arteriovenous oxygen difference

(5, 10) resulting in an increased maximal oxygen output and aerobic power (maximal oxygen uptake). In patients with coronary disease the arteriovenous oxygen difference and the cardiac work was observed at submaximal loads after training (11). It was of interest to study the effects of training in patients receiving large doses of chlorpromazine. According to a previous study (3) it is likely to lower the high blood level of noradrenaline in these patients.

The present series consists of two groups of patients. This is because as a rule patients of this type cannot manage without drugs for long periods of time. In the group given drug treatment were included who had been treated exclusively with one drug (chlorpromazine) in large doses. This also limited the number of patients available. Despite this the patients form a rather heterogeneous group. The results should therefore be interpreted with caution. In case reports of patients taking part in a physical training programme (12) there was a tendency for a decrease in cardiac output to be observed in four patients in group I and a higher after

MATERIAL

Four male schizophrenics not treated with chlorpromazine (nos 1, 2, 4 and 13 in series I) and five male patients treated with chlorpromazine (1800-3600 mg/day, nos 6, 7, 8, 9 and 10) took part in the training programme. They were selected after 1-4 months of training, except one who had been treated already because they could not

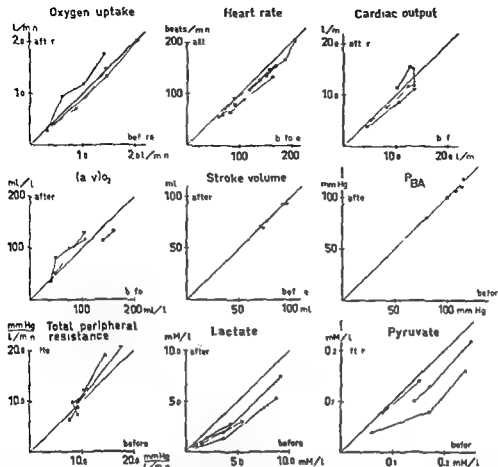


Fig 1 Group I (not treated with chlorpromazine). 'Oxygen uptake heart rate cardiac output' arteriovenous oxygen difference stroke volume brachial artery mean pressure total peripheral resistance arterial lactate and pyruvate concentrations before and after physical training.

Unfilled symbols at rest supine filled symbols during exercise in sitting position. Patient no 1 (O ●) no 2 (□ ■) no 4 (▽ ▼) no 13 (Δ ▲). The identity line is drawn.

zine. The age, height, weight of the patients as well as the doses and duration are shown in Table I in the preceding paper (4).

METHODS

The same physiological methods and procedure were used as in the preceding article (4). Oxygen uptake, cardiac output (indicator dilution technique), arterial blood pressure, arterial blood lactate, pyruvate and noradrenaline concentration were determined while the patient was at rest in the supine position and while cycling on an ergometer in the sitting position with stepwise increased work loads up to the highest which the patient could tolerate for 10–11 min.

The heart volume was determined roentgenologically in the prone position (9).

The physical training consisted of exercise on a bicycle ergometer five days a week. The patients exercised at the highest or nearly highest work load which they could maintain for six min. On each occasion the patients

cycled for three six minute periods at six minute intervals during which they often walked, played with a ball etc. The heart rate during exercise was regularly recorded and the training load was increased with increasing physical performance.

All the patients had performed a preliminary work test some days before the actual physiological studies.

RESULTS

The results obtained in group I (without chlorpromazine) and in group II (with large doses of chlorpromazine) are shown in Figs 1 and 2.

In group I the largest work load that the patients could maintain for at least 5 min (usually 10–11 min) was on the average 713 kpm/min (range 600–900) before training. After training it increased in three of the four patients and was on the average 900 kpm/min (range 750–

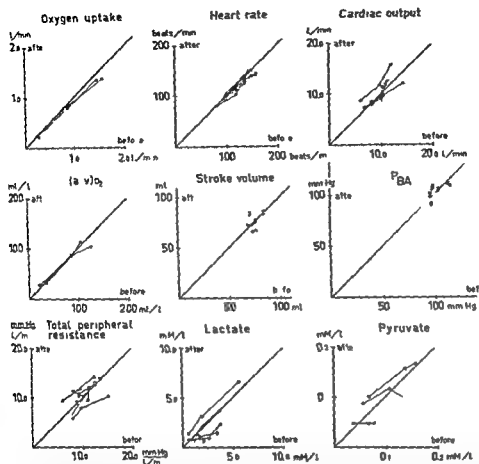


Fig 2 Group II (treated with chlorpromazine) Oxygen uptake heart rate cardiac output, arteriovenous oxygen difference stroke volume brachial artery mean pressure total peripheral resistance arterial lactate and pyruvate concentration before and after physical training. Un-

filled symbols at rest supine filled symbols during exercise sitting position Patient no 6 (●) no 7 (□) no 8 (▲) no 9 (▼) no 11 (□). The identity line is drawn.

1050). In group II the corresponding values were 600 kpm/min (for all patients) and 780 kpm/min (range 600–900). After training performance was higher in all patients except one. Mechanical efficiency did not seem to change substantially with training as is apparent from the oxygen uptake (Figs 1 and 2).

In group I with the exception of patient 13 at 300 kpm/min the heart rate was lower in all four patients both at rest and during exercise after training than before training. The average difference was at rest 10 beats/min at 300 kpm/min 8 beats/min and at 600 kpm/min 20 beats/min. No such tendency was observed at rest in group II where also the average heart rate was considerably higher than in group I (102 compared with 76 beats/min). In three of

the five patients the heart rate at 300 and 600 kpm/min was lower after training.

With the exception of patient 2 in group I and patient 6 in group II the cardiac output was smaller after than before training at the same work load—the results at the heaviest work load were then disregarded. There was a tendency (5 of 8 patients) also at rest for cardiac output to be lower after training. In three of four patients in group I and in four out of five in group II the highest values measured were higher after than before training.

In patients 1 and 13 in group I the arteriovenous oxygen difference increased after training. It decreased in patient 2 and was largely unchanged in the other two patients in group II where oxygen uptake was measured.

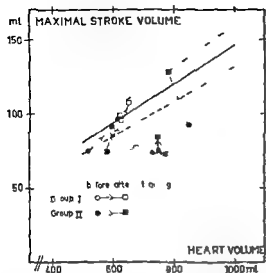


Fig 3 Maximal stroke volume during exercise in relation to heart volume in prone position. Data before and after training are connected for each individual in groups I and II. The straight line without symbols gives the relationship in healthy young men as reported by Astrand et al (1). The standard deviation is shown with broken lines.

In patients 1 and 2 training tended to increase the stroke volume. In no 1 this increase was seen only at rest. In group II this tendency was observed in no 6 and for the heaviest work load also in nos 7, 9 and 11. In two of four patients in group I and in three of five patients in group II the maximal stroke volume during exercise was larger than before training (see Fig 3). In the rest of the patients—except no 8—it was unchanged or slightly lower after training. In Fig 3 the maximal stroke volume during exer-

cise is also related to the roentgenologically determined heart volume in the prone position. The figure shows that the stroke volume tended to become normal in five of eight patients but also that the effect of training varied from patient to patient. Training had no demonstrable effect on the heart volume and the plasma and blood volumes.

In some of the patients in group II (nos 8, 9 and 11) the mean arterial pressure fell during exercise with a lower value at the highest work load than at rest in supine position (Figs 4 and 5). None of the patients in group I showed such a decrease. There was no apparent training effect.

The arterial lactate concentration was lower after than before training in all patients in group I. In group II a similar tendency was observed in patients 6, 9 and 11. The arterial pyruvate concentration also showed a similar though slighter tendency.

The noradrenaline level in arterial blood plasma (Figs 6 and 7) fell after training in all patients except in no 8. The fall in pressure was most marked in the other three subjects examined in group II where also the highest levels were found. After training patient no 8 did not show any decrease in heart rate or in lactate during exercise.

DISCUSSION

Training produced a demonstrable effect in both groups of patients but change in adaptation of circulation to exercise was more evident in the patients not treated with chlorpromazine. The daily reports on the patients' physical performance

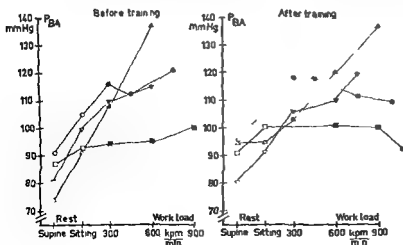


Fig 4 Brachial artery mean pressure (\bar{P}_{BA}) in group I at rest supine and sitting and during exercise. Left half of the figure shows results before and right half results after training. For explanation of symbols see Fig. 1.

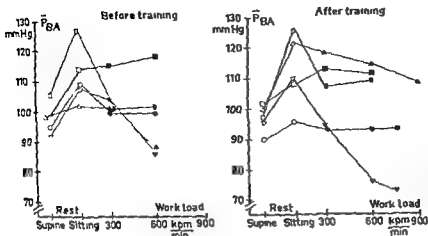


Fig 5 Brachial artery mean pressure (P_{BA}) in group II at rest supine and sitting and during exercise. Left half of the figure shows results before and right half results after training. For explanation of symbols see Fig 2

indicated also that in both groups the power of endurance and the maximal tolerable work load increased. Part of the increase in the highest work load may well have been due to neuro-muscular factors without any substantial change in the circulatory capacity.

Our findings agree in general with the results of the pilot study (2) where training was accompanied by a considerable increase in physical performance. The present study showed a decrease in heart rate and in lactate and noradrenaline concentrations after exercise. A reduced lactate concentration during exercise after training is a common finding (e.g. 5, 10, 11). It can be caused by a better relationship between

blood flow and metabolism in the working muscle at the same work load but also by a changed removal rate. It may indicate increased power of endurance and well-trained athletes have even a lower lactate concentration than untrained persons at the same relative work load (the same percentage of the maximal oxygen uptake) (7).

In the present material the decrease in heart rate with training, which is also a common finding (e.g. 5, 8, 10, 11) was not combined in all patients with identical changes in the adaptation of the circulation to exercise. Some patients had before training a hyperkinetic circulation with probably a better peripheral regulation of the

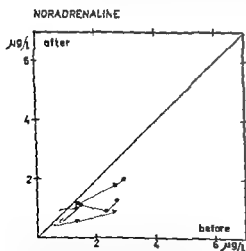


Fig 6 Noradrenaline concentration in blood plasma before and after training in group I. For explanation of symbols see Fig 1. The identity line is drawn.

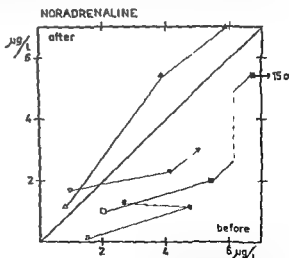


Fig 7 Noradrenaline concentration in blood plasma before and after training in group II. For explanation of symbols see Fig 2. The identity line is drawn.

circulation and a lower cardiac output after training (cf 5 11) whereas others showed an increased stroke volume with training. In some recent training studies in young healthy men more drastic effects have been achieved (5 10). Even if the training in our study must be regarded as strenuous it was still more strenuous in the investigation reported by Saltin et al (10).

The fall in noradrenaline level during exercise after training is of special interest. According to Vendsalu (12) a given work load for a given length of time elicited an approximately similar rise in the blood noradrenaline level. In his report no effect due to training was observed. In our patients however noradrenaline concentration was reduced during exercise after training both in patients treated with chlorpromazine and in those who had received no such therapy. This may be of clinical importance if the very high noradrenaline level in treated patients infers a risk of circulatory disturbances.

It is possible that the effect of training on the noradrenaline blood levels is due to the fact that trained muscles contain less lactic acid and other metabolites during work. The metabolites may cause dilatation with consequent compensatory increase of adrenergic activity. These metabolites may also have an effect on the destruction of the released noradrenaline e.g. on the re-uptake mechanism by the membrane pump. On the other hand in the chlorpromazine treated patients the high blood noradrenaline levels before training may have had metabolic effects. Thus the arterial blood lactate and noradrenaline decreased after training.

The present results and our experience during the investigation show that physical training can be successfully carried out in patients suffering from severe mental disorders who have been inactive for a long time. Treatment with large doses of chlorpromazine probably limits the effect of training on the adaptation of the circulation to exercise. The suppressive effect of training on the very high noradrenaline level in these patients seems to indicate that it is necessary that even chlorpromazine treated patients get adequate physical exercise. For these patients β receptor blocking drugs may possibly be useful for reducing the risk of heart complications in association with training.

ACKNOWLEDGEMENTS

This work has been supported by grants from the Swedish State Medical Research Council (nos. F-0166B A and 14X 166-02 and -03) the AB Leo Fund for Research and the Folksam Research Fund for Rehabilitation.

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BLOOD VOLUME AND EXCHANGEABLE SODIUM IN ESSENTIAL HYPERTENSION

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Abstract 1 No significant difference in total blood volume was demonstrated between patients with untreated essential hypertension and normotensive controls. In hypertensive women the mean blood volume was 2117 ml/m or 63.0 ml/kg as against 2259 ml/m² or 59.2 ml/kg in controls. The corresponding figures for hypertensive men were 2768 ml/m or 67.1 ml/kg and 2391 ml/m or 66.0 ml/kg for controls.

2. Exchangeable sodium was significantly increased in men with essential hypertension 1736 mEq/m or 42.4 mEq/kg while 1639 mEq/m² or 39.2 mEq/kg was found in normotensive controls. Increased exchangeable sodium was also demonstrated in hypertensive women, 1502 mEq/m or 41.2 mEq/kg in comparison with normotensive controls whose values were 1445 mEq/m² or 39.9 mEq/kg. However because of a difference in height and weight between the hypertensive and the normotensive women the results must be taken with reservation.

While a number of studies on changes in blood volume and exchangeable sodium in hypertensive patients on various antipressor regimens have been reported (2, 10, 11, 22, 23, 25) only a few standardized studies of these parameters in untreated hypertensive patients and normotensive controls have been undertaken and the results presented have often been divergent. Some investigators have found a reduced blood volume in essential hypertension (18, 19) whereas others have found that it was normal (5, 28). Similar disagreement exists in regard to exchangeable sodium where both normal (7, 17) and increased (6, 20, 27) values have been reported. Differences in the composition of the control groups could be one of the factors responsible for these contradictory results. Blood volume and exchangeable sodium expressed in ml/kg body weight decrease with increasing body weight (9, 14, 17, 24) and adipose tissue in women contains less blood than in men (1, 3, 14, 17). Blood volume

does not seem however to vary with age (4, 14, 26). If blood volume and exchangeable sodium are to be compared in different groups then these groups must be comparable in regard to height, weight and sex. Moreover other conditions which might influence these parameters such as renal insufficiency, edema, congestive heart failure and hematologic diseases must be excluded. The purpose of the present study is to compare blood volume and exchangeable sodium in patients with untreated essential hypertension with these values in normotensive controls.

MATERIAL AND METHODS

Total blood volume was measured in 22 patients (12 men and 10 women) with untreated essential hypertension and in 20 normotensives (10 men and 10 women). Exchangeable sodium was measured in 15 patients (10 men and 5 women) with essential hypertension and in 19 normotensive controls (11 men and 8 women). Only hospitalized subjects were investigated and none of them showed evidence of reduced kidney function, fluid retention, congestive heart failure or hematologic disease. All the hypertensive patients had a diastolic blood pressure over 100 mm Hg. Blood pressure and eyeground findings in the hypertensive patients are given in Tables I, III, VI and VIII. All the test subjects had been on a diet containing approximately 6 g of sodium for not less than three days and all the studies were made in the fasting state on patients who had been resting in bed for at least three hours.

Total blood volume was measured as the sum of erythrocyte and plasma volume with a double isotope dilution technique using ⁵¹Cr and ¹²⁵I-albumin and ²²Na was used in measuring 24-hour exchangeable sodium, as previously described (10, 12).

RESULTS

Blood volume

Results in ten hypertensive women (Table I) were compared with those obtained in a group of ten

Table I Blood volume in hypertensive women

Pat no	Height (cm)	Weight (kg)	Surface area (m ²)	Eye grounds	Blood pressure (mm Hg)	Red cell volume (ml)	Plasma volume (ml)	Total blood volume		
								(ml)	(ml/m ²)	(ml/kg)
1	162	50.5	1.52	II	200/110	938	2542	3480	2362	■
2	159	65.2	1.67	II	165/105	1349	2821	4370	2613	67
3	165	66.4	1.73	II	170/115	1727	3097	4824	2786	73
4	160	64.2	1.71	II	180/110	1505	2591	4096	2389	63
5	163	91.0	1.97	II	155/105	1782	2880	4662	2370	50
6	167	54.5	1.61	II	200/115	1473	2315	3786	2358	69
7	155	59.5	1.56	II	160/100	1361	2156	3517	2258	59
8	153	54.7	1.51	II	170/105	1122	1891	3013	1996	56
9	155	52.5	1.50	II	170/110	1019	2036	3055	2040	58
10	154	53.8	1.51	III	200/105	1087	1916	3003	1993	56
Mean	159 ■	61.2	1.63			1356	2425	3781	2317	63.0
±s.d.	5.3	11.9	0.15			301	426	685	240	7.4
Coefficient of variation									10.3	11.7

normotensive control patients (Table II). Average height, weight and surface area are almost identical in the two groups. Body surface was calculated using Du Bois formula (8) and blood volume is given as total ml/ml/m² surface area and ml/kg body weight. Blood volume averaged 58 ml/m² or 3.8 ml/kg more in the hypertensive women than in the controls. This difference however is not significant.

Tables III and IV give the results in 12 hypertensive and ten normotensive men. Here too average height, weight and surface area are almost identical in the two groups. Blood volume in the hypertensives averaged 177 ml/m² or 1.1 ml/kg more than in the controls, but the difference is not significant. It can be seen from

Tables I, II, III and IV that blood volume in ml/m² is a better parameter than blood volume in ml/kg, as the coefficient of variation is a slightly lower for ml/m². Table V gives the mean value for blood volumes found in the present study together with those reported by other investigators. Individual values in ml/m² and ml/kg are illustrated in Fig. 1. Here it can be seen that blood volume in men is on the average larger than in women, especially if expressed in ml/m².

Exchangeable sodium

Results in five hypertensive and seven normotensive women are shown in Tables VI and VII. Unfortunately it was not possible to obtain two

Table II Blood volume in normotensive women

Pat. no	Height (cm)	Weight (kg)	Surface area (m ²)	Red cell volume (ml)	Plasma volume (ml)	Total blood volume		
						(ml)	(ml/m ²)	(ml/kg)
1	160	62.7	1.65	1306	2560	3866	2339	67
2	163	63.4	1.68	1376	2939	4315	2564	68
3	161	58.5	1.61	1000	2188	3188	1978	54
4	161	57.3	1.60	1502	2556	4058	2544	71
5	149	51.0	1.44	1040	1831	2871	1997	56
6	161	70.0	1.74	1367	2317	3684	2431	53
7	170	62.5	1.72	1308	2842	4150	2090	65
8	154	58.8	1.56	1305	2282	3587	2293	61
9	160	65.3	1.68	1309	2202	3511	2256	49
10	160	64.4	1.67	1208	2181	3389	2027	53
Mean	159.9	61.2	1.63	1273	2390	3663	2259	59
±s.d.	5.4	5.2	0.09	154	335	449	221	7.3
Coefficient of variation								9.9

Table III Blood volume in hypertensive men

Pat. no	Height (cm)	Weight (kg)	Surface area (m ²)	Eye grounds	Blood pressure (mm. Hg)	Red cell volume (ml)	Plasma volume (ml)	Total blood volume		
								(ml)	(ml/m ²)	(ml/kg)
1	168	73.8	1.86	II	220/110	1761	2284	4645	2675	62
2	191	90.5	2.20	I-II	160/110	2240	3516	5756	2621	67
3	180	100.0	2.20	II	180/105	2422	3531	5953	2711	59
4	183	87.5	2.12	II	175/110	2425	3492	5917	2797	68
5	169	81.5	1.94	II	160/110	2138	2987	5145	2649	62
6	177	80.6	1.98	II	175/120	2192	3449	5641	2851	60
7	176	73.5	1.89	II	195/110	2326	3559	5885	3106	80
8	160	60.0	1.62	II	165/110	2096	2890	4986	3074	70
9	170	77.2	1.89	II	175/115	1878	2638	4516	2393	58
10	160	59.3	1.61	II	215/115	1825	3062	4887	3028	88
11	173	74.8	1.89	II	180/125	1817	3419	5236	2777	68
12	172	67.7	1.80	III	230/120	1591	2968	4559	2533	68
Mean	173.4	77.5	1.92			2061	3200	5260	2768	67.1
±s.d.	9.2	15.9	0.19			309	323	551	219	8.1
Coefficient of variation, %									7.9	12.2

Table IV Blood volume in normotensive men

Pat. no	Height (cm)	Weight (kg)	Surface area (m ²)	Red cell volume (ml)	Plasma volume (ml)	Total blood volume		
						(ml)	(ml/m ²)	(ml/kg)
1	176	64.3	1.79	1772	2868	4640	25.9	73
2	176	72.0	1.88	2113	3415	5528	29.43	76
3	177	74.5	1.91	1817	2694	4511	23.58	61
4	170	69.1	1.80	1790	2794	4584	25.47	88
5	174	71.3	1.85	1936	2862	4798	25.87	67
6	171	76.0	1.88	1960	2971	4931	26.20	65
7	171	83.5	2.01	2181	4025	6206	30.91	70
8	164	68.8	1.75	1515	2340	3855	22.08	56
9	179	93.0	2.23	2143	3285	5428	24.34	38
10	179	93.5	2.12	1975	3395	5370	25.27	57
Mean	173	77.1	1.92	1920	3063	4983	25.91	65.1
±s.d.	7.4	10.6	0.15	207	475	663	2.39	7.3
Coefficient of variation							9.9	11.2

Table V Blood volume (mean value) in hypertensive and normotensive patients

	Blood volume							
	(ml/m ²)				(ml/kg)			
	Hypertensive		Normotensive		Hypertensive		Normotensive	
	♀	♂	♀	♂	♀	♂	♀	♂
Present series	317	2768	259	2591	63.0	67.0	59.2	66.0
Rochlin et al. 1960	—	—	—	—	61.0	67.3	70.0	76.1
	♀ + ♂		♀ + ♂		♀ + ♂		♀ + ♂	
Reilly et al. 1964	2370		2490		65.3		65.5	
Walker et al. 1956	—		5.0		58.4		63.1	

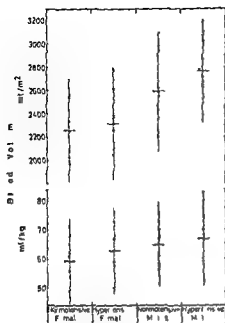


Fig 1 The relation of blood volume to weight and surface area in normotensive and hypertensive men and women. The horizontal line represents the mean value for all patients the vertical line gives ± 2 SD.

fully comparable groups. The hypertensive patients weighed on the average 2.7 kg less than the normotensives and were 1.7 cm shorter. The relationship between height and weight was however almost identical for the two groups. Exchangeable sodium averaged 1502 ± 126 mEq/m for the hypertensives and 1445 ± 78 mEq/m for the controls. Expressed in mEq/kg the figures are 41.2 ± 1.8 and 38.9 ± 1.6 . Thus the hypertensive women had an exchangeable sodium that was 57 mEq/m greater than that of the controls; the difference was not, however sig-

nificant (SE 50 $p > 0.2$). The difference in mEq/kg was just on the borderline of significance (SE 11 $0.05 < p < 0.1$). But because of the above mentioned difference in height and weight between the two groups comparisons between them must be taken with reservation.

Tables VIII and IX show exchangeable sodium in ten hypertensive and 12 normotensive men. The difference in height and weight is so small that comparison is possible. Exchangeable sodium was 1736 ± 95 mEq/m in the hypertensive patients and 1639 ± 111 mEq/m in the controls. This difference of 97 mEq/m is significant (SE 47 $p < 0.05$). Expressed per kg body weight the figures are 42.4 ± 4.9 and 39.2 ± 2.0 and the difference of 3.2 is also significant (SE 1.5 $p < 0.05$). As can be seen in Table VIII the coefficient of variation was lower for exchangeable sodium in mEq/m than per kg in the hypertensive men, indicating that exchangeable sodium in mEq/m is the better parameter. The opposite is true in the other groups studied (Tables VI, VII and IX). Fig 2 illustrates individual values for exchangeable sodium in mEq/m and per kg in both hypertensive and control patients. Most notable here is the fact that men have on the average a larger amount of exchangeable sodium than women.

DISCUSSION

In the present study no significant difference in blood volume was observed between hypertensive patients and normal controls. Comparison with results obtained by other investigators is difficult, particularly because of different way of selecting

Table VI. Exchangeable sodium in hypertensive women

Pat no	Height (cm)	Weight (kg)	Surface area (m ²)	Eye grounds	Blood pressure (mm Hg)	Exchangeable sodium		
						(mEq)	(mEq/m ²)	(mEq/kg)
1	159	65.2	1.67	II	165/105	2856	1707	43.8
2	161	63.5	1.67	II	170/110	2357	1532	40.3
3	154	50.5	1.47	III	170/125	2101	1429	41.6
4	153	52.5	1.50	II	170/100	2185	1459	41.6
5	154	53.8	1.51	III	205/105	2085	1384	38.8
Mean	156.8	57.1	1.56			2357	1502	41.2
\pm SD	3.2	6.7	0.10			244	126	1.8
Coefficient of variation							8.3	4.3

Table VII *Exchangeable sodium in normotensive women*

Pat. no	Height (cm)	Weight (kg)	Surface area (m ²)	Exchangeable sodium		
				(mEq)	(mEq/m ²)	(mEq/kg)
1	164	58.5	1.63	2406	1473	41.1
2	160	62.7	1.65	2476	1498	39.9
3	161	58.5	1.61	2256	1399	38.6
4	149	51.0	1.64	2061	1433	40.0
5	160	65.3	1.68	2335	1388	39.9
6	154	58.5	1.56	2343	1498	35.7
7	160	64.4	1.67	2381	1425	37.0
Mean	158.3	59.8	1.63	2322	1445	38.9
±s.d.	5.1	4.8	0.04	134	78	1.6
Coefficient of variation					5.4	4.1

Table VIII *Exchangeable sodium in hypertensive men*

Pat. no	Height (cm)	Weight (kg)	Surface area (m ²)	Eye-grounds	Blood pressure (mm Hg)	Exchangeable sodium		
						(mEq)	(mEq/m ²)	(mEq/kg)
1	191	90.5	2.20	I-II	160/110	3705	1687	40.9
2	180	100.0	2.20	II	180/105	3683	1678	36.8
3	185	87.5	2.12	II	175/110	3705	1752	42.3
4	169	83.5	1.94	II	160/110	3253	1675	39.0
5	170	77.2	1.89	II	175/115	3093	1634	39.9
6	160	59.3	1.61	II	215/115	3101	1921	52.3
7	173	74.8	1.89	II	180/125	3710	1703	42.9
8	175	76.0	1.91	III	220/120	3626	1895	47.7
9	173	100.8	2.14	III	195/145	3676	1718	36.5
10	172	67.7	1.80	III	230/120	3059	1700	45.5
Mean	174.8	81.7	1.97			3410	1736	42.4
±s.d.	8.7	13.4	0.19			290	95	4.9
Coefficient of variation							5.4	11.3

Table IX *Exchangeable sodium in normotensive men*

Pat. no	Height (cm)	Weight (kg)	Surface area (m ²)	Exchangeable sodium		
				(mEq)	(mEq/m ²)	(mEq/kg)
1	171	76.0	1.88	3150	1674	41.4
2	171	88.5	2.01	3588	1787	40.5
3	164	68.8	1.75	2603	1487	37.8
4	170	74.4	1.89	3067	1624	39.6
5	163	59.8	1.64	2487	1515	41.0
6	178	68.9	1.86	2935	1579	42.6
7	169	94.7	2.05	3549	1737	37.5
8	172	81.7	1.64	2957	1517	36.2
9	179	93.5	2.1	3484	1640	37.3
10	180	100.5	2.20	3950	1796	39.3
11	197	93.0	2.73	3485	1563	37.5
12	178	96.3	2.14	3761	1759	39.1
Mean	173.9	83.0	1.95	3231	1639	39.2
±s.d.	7.9	13.2	0.71	457	111	2.0
Coefficient of variation					6.7	5.1

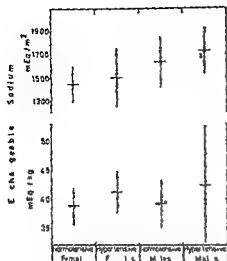


Fig 2 The relation of exchangeable sodium to weight and surface area in normotensive and hypertensive men and women. The horizontal line represents the mean value for all patients; the vertical line gives ± 2 SD.

controls. Harris and Gibson (13) found that blood volume was normal and Rusznayak (21) that it was reduced in essential hypertension. These investigators, however, compared the values obtained in hypertensives with theoretical values derived from a nomogram. Reilly et al (18) studied 17 men and women with various cardiac diseases without signs of incompensation. Eight of these patients were hypertensive. They found a significantly lower blood volume in all 17 patients compared with a control group of 89 men (Table V); however, no information was given on the height and weight of the subjects studied. Rochlin et al (19) also observed a significantly lower blood volume in patients with essential hypertension compared with controls (Table V) but the hypertensive women weighed on the average 5 kg more than the controls in spite of similar height and the hypertensive men weighed 2 kg more and were 2.5 cm shorter than the average for the controls. On the whole, the hypertensive patients reported on by Rochlin et al were more obese than the controls, which could at least in part explain the lower blood volume in the hypertensive patients. Walker et al (28) found an average blood volume of 58.4 ml/kg in a group of hypertensive men and women as compared with 63.1 ml/kg in control patients (Table V). These investigators concluded, however, that the difference was not significant.

Their hypertensive patients were also more obese than the controls.

In the present study exchangeable sodium in both mEq/m² and mEq/kg was found to be significantly increased in hypertensive men. Exchangeable sodium in mEq/kg was likewise increased in hypertensive women but as mentioned above, direct comparison between the two groups of women, hypertensives and controls, is difficult because of differences in average height and weight. Similar results were reported by Tomimaga (27) who found that average exchangeable sodium was 46.8 mEq/kg in 36 hypertensive men and 37.2 mEq/kg in seven normotensive controls. Corresponding values were 45.1 and 38.0 respectively in 15 hypertensive women and in three controls. Moreover, this author reported that exchangeable sodium increased with the increasing severity of hypertension. Ross (20) found that average exchangeable sodium was 43.7 mEq/kg in a group of hypertensive men and women with grade I to II eyegrounds as against 38.1 mEq/kg in normals. In severe hypertension with grade III to IV eyegrounds, the values for exchangeable sodium were much higher. Dahl et al (6) demonstrated a larger sodium pool and increased biological half life for ^{22}Na in hypertensive patients compared with controls. On the other hand, De Graeff (7) and Moore et al (17) observed no difference in exchangeable sodium between hypertensives and normotensives. It is not possible to explain this discrepancy. It is a well known fact, however, that sodium plays an important role in the pathogenesis of hypertension. Rigid salt restriction often leads to a fall in arterial blood pressure, whereas too large a sodium intake produces an increase in blood pressure (6, 7, 15). However, as emphasized by Ross (20) and Dahl (6), it is most likely that increased exchangeable sodium is a consequence rather than a cause of hypertension.

ACKNOWLEDGEMENTS

This investigation was aided by grants from Statens Almindelige Videnskabsfond and from Carlsberg's Fond.

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PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA

A Clinical Study

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Abstract Case reports of eight patients with the PNH defect are presented.

The diagnostic criteria applied are discussed. Ham's and Crosby's tests, the sucrose haemolysis test, acetylcholine esterase activity of the red cells and signs of intravascular haemolysis with emphasis on the determination of lactate dehydrogenase activity in blood and bone marrow.

The diversity of the presentation of PNH is outlined. One patient presented with attacks of abdominal pain for two years before diagnosis; three patients had long-standing pancytopenia with few clues to the diagnosis; two patients presented as cases of aplastic anaemia with initially negative Ham's tests and two patients had concomitant myelofibrosis. The relationship of PNH to other blood diseases is discussed and a possible connection with myelofibrosis is suggested. It thus appears that the PNH erythrocyte defect is encountered not only in the clinically classical PNH syndrome but also in a variety of pancytopenias which are difficult to classify and finally in some better defined conditions such as aplastic anaemia and myelofibrosis. This could be interpreted in several ways: 1. PNH is a nosological entity with an extremely wide clinical spectrum; 2. PNH is not a nosological entity but erythroid cell lines carrying the PNH defect may be set up under many conditions when haemopoiesis is afflicted by a primary haematological disorder; 3. PNH exists (a) as a primary disorder, the clinically classical PNH syndrome and (b) as a secondary defect in a variety of other haematological disorders. It is not yet possible to determine which of these possibilities, if any, is the right one, although at the present time the second interpretation would appear to be the most likely one.

Clinical features are mentioned particularly hypercoagulability and the occurrence of thromboses. An interesting aspect is that the majority of patients had an abnormal EEG. The significance of this remains to be determined. The multiple mechanism of anaemia in PNH is emphasized, including marrow hypoplasia, haemolysis, iron deficiency, autoimmunity and folic acid deficiency.

The value of blood transfusions, iron, adrenocorticoid and anabolic steroids in the management of PNH is discussed.

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare haemolytic anaemia due to a largely unknown erythrocyte defect. In the classical form the disease is characterized by chronic intravascular haemolysis with occasional attacks of accentuated haemolysis during sleep which may result in haemoglobinuria.

Excellent reviews have been given by Dacie (10) and Crosby (6) and recently reviews concerning pathogenetic aspects of the disease have been published by Hartmann and Jenkins (19) and Firkin (15) and Hinz (24).

This paper contains the case histories of eight patients with the PNH defect which emphasize that the PNH defect occurs in conditions which clinically differ widely. A point of particular interest is the occurrence of PNH red cells in myelofibrosis.

METHODS AND CASE REPORTS

The erythrocyte acetylcholine esterase activity has been determined as described by Jørgensen (26). The normal range of activity in our laboratory is $16.0-32 \mu\text{M}/\text{min/g}$ haemoglobin. The serum lactate dehydrogenase activity has been determined as described by Laursen (7,8). The normal range of activity being $7-23 \mu\text{M}/\text{h}/\mu\text{l}$. Lactate dehydrogenase isoenzymes have been determined as described by Wieme (34) and van der Helm (22) and the bands of activity have been designated 1-5, the most anodic having the lowest number. Coagulation tests have been carried out in the Coagulation Laboratory as described in the following article (1). The sucrose haemolysis test was carried out as described in a following paper (18). The normal range for MCV in our laboratory is 81-109.

Patient no 1 (RH A 18 526)

Farmer born in 1911. There was no family history of blood disease nor was there any exposure to drugs or chemicals except as is customary in agriculture. Prev-

ously the patient had been well. In 1958 he began experiencing attacks of abdominal pain. The attacks never started during sleep and were never accompanied by the voiding of dark or red urine. At the local hospital no cause of the abdominal pain was found, and during the next two years the patient often received morphine for relief of pain. In 1960 haemoglobinuria was found in connection with an attack of pain, and the diagnosis of PNH was made.

In 1962 the patient was admitted to this department. Physical examination was normal. Haemoglobin was 9.5 g/100 ml, leucocytes and thrombocytes were normal. Reticulocytes were 2.2%, plasma haaptoglobin 0, plasma haemoglobin 28 mg/100 ml and serum bilirubin 11.16 mg/100 ml. There was intermittent haemoglobinuria. Hama test was positive, Crosby's test negative, Coombs direct test, Donath-Landsteiner's test and a Wassermann test were all negative. Osmotic fragility was slightly increased, a blood smear revealed no spherocytic or elliptocytes. Haemoglobin electrophoresis was normal. Serum iron was 27 μ g/100 ml and TIBC 353 μ g/100 ml. MCV 96, MCHC 29. Roentgenologic studies of the intestinal tract were normal; there was no blood in the stools and no sign of liver disease.

A diagnosis of PNH with secondary iron deficiency anaemia was made and the patient was treated with oral iron, following which the haemoglobin concentration rose to 11.1 g/100 ml.

The patient continued to have attacks of severe abdominal pain. Treatment at home with low molecular weight dextran had no effect. The patient was last seen in this department in September 1966. As before the patient had slight haemolytic anaemia with a positive Hama test. The sucrose haemolysis test was positive, serum LDH was 106 units (four times the normal value) with raised values for isoenzymes 1 and 2. Plasma haemoglobin was raised, with accentuated values during an ep. Acetylcholine esterase in the erythrocytes was slightly decreased (13.4 units).

In the hope of demonstrating a hypercoagulable state as a background to the repeated attacks of abdominal pain, coagulation tests were made. The tests performed at a time of no abdominal pain, were normal except for slightly increased thrombin generation. One night however the patient experienced the usual abdominal pain—the first time in the eight year course of his disease when the pain occurred during sleep—and haemoglobinuria was found. Coagulation tests during this attack revealed a state of pronounced hypercoagulability with short coagulation time and accelerated thromboplastin generation. This state of hypercoagulability lasted for about two days after which the state of coagulation again became as shown by the first tests. The patient was put on treatment with phenprocoumon (Marcoumar[®]) in the hope of reducing the severity of the abdominal pain and was discharged from the hospital. This treatment, however had no effect. The patient is reported (June 1967) to suffer from unchanged attacks of abdominal pain as before.

Summary Middle aged man who for two years had attacks of abdominal pain before the diag-

nosis of PNH was made. The patient developed secondary iron deficiency anaemia which responded well to iron medication. During an attack of abdominal pain a hypercoagulable state was demonstrated.

Patient no 2 (RH 4.21.651)

Woman, labourer born in 1909. The patient's aunt was reported to have suffered from a blood disease treated with a liver preparation but otherwise there was no family history of blood disease. No known exposure to drugs or chemicals. Except for fatigue from which she had suffered since childhood she had been well until 1940 when she one day experienced pain in the abdomen and voided red urine. Since then the patient has never experienced similar attacks. In the same year she was admitted to hospital because of pancytopenia and haemolytic anaemia and in the period 1940-65 she was hospitalized several times for the same reason. Her complaints were those of tiredness and nervousness. On every occasion pancytopenia and haemolytic anaemia were found but the aetiology was never clarified. Hama and Crosby's tests were not made.

In January 1965 she had a sudden attack of paresis of the right extremities, difficulty in speech and inability to walk. An electroencephalogram was abnormal with spikes and some 6-7 Hz activity with a left sided pre dominance. The patient quickly recovered but because of pancytopenia she was transferred to the present department in February 1965.

Physical examination was normal. Haemoglobin was 9 g/100 ml, leucocytes 3000/ μ l and thrombocytes 167 000/ μ l. Reticulocytes were 4.8-4.6% in the absence of demonstrable haemorrhage. The bone marrow was hyperplastic with stainable iron. Serum iron 94 μ g/100 ml, TIBC 234 μ g/100 ml, MCV 106, MCHC 3. Haaptoglobin 4 mg/100 ml. Serum folic acid and serum B¹² were normal. Hama test was positive, Crosby's test negative, the acetylcholine esterase activity in the erythrocytes low (13.5 units). Coombs direct test, Donath-Landsteiner's test, a Wassermann test, osmotic fragility and haemoglobin electrophoresis were all normal. An autosurvival study with chromium-51 tagged erythrocytes showed a half life of 20 days with no evidence of a double population. Ferrokinetic studies with radioisotopes indicated ineffective erythropoiesis (plasma iron clearance, half time 36 min, plasma iron turnover 1.28 mg/74 h, Fe⁵⁹ red cell incorporation at 11 days 58%). Treatment with prednisone 15 mg daily was initiated but as no effect was seen after one month, prednisone was discontinued.

Since the initial admission to this department the patient has been seen on several occasions. The haemolytic anaemia remains unchanged and there is still leucopenia but no thrombopenia. The patient's complaints are extreme tiredness, feeling of heaviness in the right side of the body, subternal oppression, and occasional right abdominal pain. She has not noticed the voiding of dark or red urine but haemoglobinuria has been found on several occasions. She was last seen in May 1967. The haematological parameters were unchanged except that

now some evidence of iron deficiency was found in contrast to previous studies stainable iron could no longer be demonstrated in the bone marrow. The MCV had decreased from 106 to 99 although the MCHC was still normal. Serum iron and TIBC were both low because of concomitant infection. The sucrose haemolysis test was positive serum LDH 100 with raised values for isoenzymes 1 and 2 plasma haemoglobin 10 mg/100 ml with raised values during sleep. Coagulation tests were normal. The EEG was unchanged.

Summary Middle aged woman who for at least 25 years has had haemolytic anaemia and pancytopenia of unknown aetiology. The period of disease apparently started with an attack of abdominal pain and the voiding of red urine but except for this attack similar events have never occurred. In 1965 the patient had an apoplectic insult, and in the same year the PNH diagnosis was made. Treatment with prednisone was of no effect. Signs of iron deficiency developed.

Patient no 3 (RH A 20 139)

Housewife, born in 1885. There was no family history of blood disease nor any known exposure to drugs or chemicals. Except for heavy otosclerosis she had previously been well.

Already in 1947 pancytopenia was found. During a stay at a local hospital to which the patient was admitted because of dyspnoea and cutaneous bleeding the following values were found: haemoglobin 31, leucocytes 1900/ μ l, thrombocytes 4900/ μ l, reticulocytes 1-3, colour index 1.3-1.6, ESR 44-83 mm/h. Haemoglobinuria was not demonstrated. No cause of the pancytopenia was found and treatment with folic acid and a liver preparation was without effect. Several transfusions with whole blood were given. After one of them an explained fever developed. During the following years the patient apparently did well and was not admitted to hospital. In 1961 she was admitted to a dermatological hospital because of a post thrombotic ulcer of the leg. Again pancytopenia was found and the patient was transferred to a medical department of the local hospital. Haemoglobin was 7.8 g/100 ml, leucocytes 1900/ μ l, thrombocytes 10 000/ μ l and reticulocytes 15-20%. For the first time during her illness Ham's and Crosby's tests were made both were slightly positive. There was still no haemoglobinuria.

In 1963 the patient was admitted to the present department. Her complaints were still only those attributable to anaemia. Except for obesity and a chronic ulcer of the leg, physical examination was normal. Haemoglobin was 8.2 g/100 ml, leucocytes 1400/ μ l, thrombocytes 64 000/ μ l. The anaemia was haemolytic, reticulocytes 20-30% with no signs of bleeding or blood regeneration, serum haptoglobin 0, plasma haemoglobin 7-10 mg/100 ml. An erythrocyte auto survival study with chromium 51 tagged cells showed a half life of 19 days with no evidence of a double erythrocyte population. Ham's test was positive, Crosby's test negative.

haemoglobinuria could not be demonstrated. Coombs direct test, Donath Landsteiner's test and a Wassermann test were all negative. Osmotic fragility was normal. A blood smear revealed no spherocytes or elliptocytes. Serum iron was 59 μ g/100 ml, TIBC 268 μ g/100 ml, MCV 107, MCHC 30.

A diagnosis of atypical PNH was made. Treatment with prednisone 70 mg a day was initiated and the patient was discharged for further control in the outpatient department. The patient however did not show up until one year later when she was readmitted because of ulcer of the leg. During this year the patient had continuously taken the prescribed prednisone. On this treatment haemoglobin had risen to 17.1 g/100 ml and there was only slight reticulocytosis (1%). Leucocytes were 3000 and thrombocytes 90 000/ μ l. Ham's and Crosby's tests were now negative. The patient felt completely well and she was discharged on prednisone 10 mg daily. Unfortunately it proved impossible to keep contact with the patient but she continued to take the prednisone as prescribed. In 1966 she was admitted to another hospital because of gangrene of a leg. From the records of the hospital it appears that the haematological values were still higher than the values prior to the initiation of prednisone treatment (haemoglobin 10.1 g/100 ml, leucocytes 3300-6000/ μ l, thrombocytes 18 000-113 000/ μ l). She died in 1966 of acute renal failure following amputation of a leg. Renal venous thrombosis was suspected but no autopsy was performed.

Summary Old woman who for at least 19 years had pancytopenia and haemolytic anaemia. Ham's test was not performed until 1961. 14 years after pancytopenia had first been demonstrated and was then positive. Ham's test was positive also in 1963 but in 1964—after one year of prednisone treatment—it had become negative while the haemoglobin concentration had risen to almost normal levels where it remained until her death in 1966. The patient's complaints were only those of anaemia. Haemoglobinuria was never demonstrated.

Patient no 4 (RH A 22 655)

Housewife, born in 1906. There was no family history of blood disease nor any known exposure to drugs or chemicals.

Since childhood the patient has had a tendency to anaemia, for which she has often been treated with iron. In 1955, 1956 and 1959 she was admitted to hospital because of anaemia. On all these occasions pancytopenia and haemolytic anaemia were found but the aetiology was never elicited. Ham's and Crosby's tests were not performed. The patient's complaints were those of anaemia and dermal infections. Treatment with vitamin B₁₂ and folic acid had no effect.

In May 1966 the patient like the rest of her family suddenly fell ill with fever, nausea, vomiting and dizziness. The rest of the family quickly recovered but three

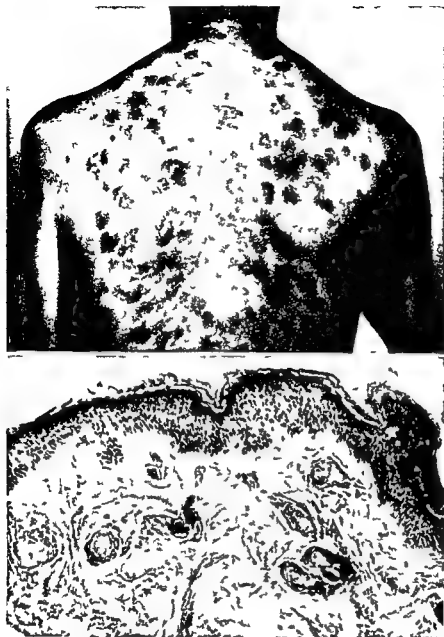


Fig 1 Patient no 4 Affected skin and corresponding biopsy with thromboses in capillaries and arterioles suggestive of thrombotic thrombocytopenic purpura

days after the onset of the disease the patient developed red and blue spots universally on the skin and shortly afterwards pain in the joints and muscles. Treatment with Ampicillin had no effect, and therefore the patient was admitted to the present hospital.

Physical examination was normal except that her skin was covered with reddish blue spots (Fig 1). The temperature was around 39°C. Haemoglobin was 81 g/100 ml, leucocytes 7200/ μ l and thrombocytes 65 000/ μ l. Reticulocytes were 1.2–2.1%. Although no bleeding could be demonstrated the haemoglobin concentration fell from 81 to 66 in four days. The bone marrow was hyperplastic with no stainable iron. Serum iron 61 μ g/100 ml, TIBC 335 μ g/100 ml, MCV 176, MCHC 33.

There was haemosiderinuria but no haemoglobinuria. Ham's test was positive, Crosby's test negative. The acetylcholine esterase in the erythrocytes was low (10.3 units). A direct Coombs test was slightly positive and the antinuclear factor test was ++. Donath Landsteiner's test and a Wassermann test were negative. Osmotic fragility was slightly increased.

A biopsy from the affected skin showed changes compatible with thrombotic thrombocytopenic purpura (Fig 1). There was however no sign of renal involvement, and neurological examination was normal. An electroencephalogram was moderately abnormal with diffuse admixture of 3–5 Hz activity. Coagulation tests showed pronounced hypercoagulability with shortened coagula-

tion time accelerated thrombin generation and increased concentrations of factors II+VII V and VIII

The patient was considered to suffer from PNH with acute immunological complications, and on this assumption treatment with prednisone 80 mg daily was initiated. The response was striking: the patient became afebrile, the haemoglobin leucocytes and thrombocytes rose to normal values, the Coombs test became negative and the cutaneous changes disappeared. The patient was discharged on oral iron and prednisone 15 mg daily.

The patient was again admitted to this department in December 1966 for a check up. She had no complaints and all reported that she felt better than during the past 15 years. Haemoglobin, leucocyte and thrombocyte values were normal; there were still signs of haemolysis with reticulocytes 6.6-6.8% and a serum LDH of 58 units (twice the normal upper value); the isoenzyme pattern was compatible with haemolysis (raised values for isoenzymes 1 and 2). Plasma haemoglobin was 10 mg/100 ml with increases during physical activity but not during sleep. The concentrations of coagulation factors were increased as during her first admission. Skin lesions were absent. The patient is still well and in complete haematological remission on a moderate dose of prednisone and oral iron. She has never had attacks of abdominal pain with red or dark urine and haemoglobinuria has never been demonstrated.

Summary Middle aged woman who for at least 11 years has had pancytopenia and haemolytic anaemia. In connection with a febrile disease the patient developed skin lesions histologically compatible with thrombotic thrombocytopenic purpura and a transiently positive Coombs test. At the same time evidence of a PNH defect was found. On treatment with prednisone the symptoms vanished and complete haematological remission developed. Haemolytic anaemia with a positive Ham's test persists but clinically the patient is completely well.

Patient no 5 (RHA 18497)

Girl born in 1949. There is no family history of blood diseases.

During the period 1952-1957 the patient was treated with large amounts of penicillin because of dermat infections. Since the age of four the patient was often treated with iron because of recurrent anaemia and she had a pronounced tendency to develop blue marks in the skin and nose bleeding. In 1955 the patient had a concussion of the brain and since then has had frequent attacks of headache of the hemicranial type for which she has been treated with dehydroergotamine.

In January 1967 the patient suddenly developed high fever of unknown aetiology. Two days later petechiae developed and the patient was admitted to the local hospital. Pancytopenia with a hypoplastic bone marrow was found. On the diagnosis of aplastic anaemia treatment was instituted with prednisone and androgen.

(Dianabol®) and vitamin B₁₂. The leucocyte count rapidly became normal but the haemoglobin and the thrombocyte count did not improve and therefore the patient was admitted to this department in May 1967.

Physical examination was normal. Haemoglobin was 8.6 g/100 ml, thrombocytes 32 000/ μ l, the leucocyte count normal. Reticulocytes were 3.0%, serum bilirubin 1.1 mg/100 ml. No bleeding could be demonstrated. Ham's and Crosby's tests were negative and there was no haemoglobinuria. Coombs direct test, Donath Landsteiner's test and a Wassermann test were all negative. Osmotic fragility was slightly increased. There were no sphaero- or elliptocytes in the blood smear. Haemoglobin electrophoresis was normal.

A diagnosis of hypoplastic anaemia was made and treatment with prednisone 25 mg daily and nortestosterone 25 mg a week was given. There was no striking response to this treatment and severe thrombocytopenia continued. Splenectomy was performed in October 1964. The spleen weighed 150 g and was microscopically normal. After splenectomy the haemoglobin concentration rose to a higher level than before the operation but the thrombopenia was unchanged. As the patient became strongly cushingoid prednisone was discontinued but this was followed by a decrease in haemoglobin from 10.9 to 7.4 g/100 ml in one month. Therefore prednisone treatment was resumed after which the haemoglobin concentration increased again. The thrombocyte count was continuously low and intermittently haemorrhagic diathesis, often as haematuria, was present. Ham's and Crosby's tests were still negative.

In March 1966 the patient was readmitted because of decreasing haemoglobin concentration. The haemoglobin was 8 g/100 ml, thrombocytes 42 000/ μ l, leucocytes normal. Reticulocytes were 4.0%. For the first time during her illness Ham's and Crosby's tests were positive. Haemosiderinuria but no haemoglobinuria was present. The acetylcholine esterase activity in the erythrocytes was low (13.5 units). Her symptoms were still only those of anaemia and thrombocytopenia. The dose of prednisone was increased but the haemoglobin concentration continued to fall. Six months later the patient was readmitted. She had now developed the full picture of PNH: attacks of abdominal pain accompanied by haemoglobinuria and chronic intravascular haemolysis. Ham's test was positive, Crosby's test negative, the sucrose haemolysis test positive. Plasma haemoglobin was only 2 mg per 100 ml but with accentuated values during sleep. Serum LDH was 98 units with raised values for isoenzymes 1 and 2. Coagulation tests showed accelerated thrombin generation and thromboplastin generation and short cephalin time. During a haemolytic attack the patient developed an accentuated degree of hypercoagulability with an accelerated thromboplastin generation time, short cephalin time and now also raised values for factors V and VIII. During sleep the EEG was normal but upon photo-stimulation unusual spikes were seen. Haemoglobin decreased to 6.1 g/100 ml. Prednisone which earlier in the disease had been given with effect was increased to 40 mg daily but the haemoglobin concentration was unaffected. At this time the serum iron was only 1.6/100 ml in spite of severe haemolysis. TIBC 345 μ g; 100 ml MCV 116 MCHC 19 and there were few

sideroblasts in the marrow. Iron deficiency was suspected and the patient was put on oral iron medication while at the same time the prednisone dose was reduced gradually. Over the next few months this resulted in an increasing haemoglobin concentration. In the middle of May 1967 the haemoglobin concentration was 9.6 g/100 ml the MCHC 31 and the patient still seems to be improving.

Summary. Young girl who in 1962 developed aplastic anaemia. Treatment with prednisone, anabolic steroid and splenectomy resulted in partial remission though the thrombocytopenia was largely unaffected. In 1966 the haemoglobin concentration decreased considerably and at this time Ham's test—negative at the onset of disease—became positive. Six months later the patient had developed the full picture of PNH. Iron deficiency developed and medication with oral iron resulted in a significant increase of haemoglobin.

Patient no 6 (RH A 20 050)

Farmer born in 1938. There was no history of blood disease in the family nor was there any exposure to drugs or chemicals except as is customary in agriculture. The patient had been well until the present illness started in October 1967 with symptoms of anaemia and cutaneous haemorrhages. At the local hospital pancytopenia was found, the bone marrow was hypoplastic. On the diagnosis of aplastic anaemia treatment with prednisone 30 mg daily was started and several transfusions with whole blood were given.

The patient was first admitted to this department in January 1963. Physical examination was normal. Haemoglobin was 5.7 g/100 ml, leucocytes 1700–1900/ μ l, thrombocytes 54 000–19 000/ μ l, and reticulocytes 1–16. A biopsy from the iliac crest showed a hypoplastic marrow. An autosurvival study with chromium 51 erythrocytes demonstrated a half life of 16 days without evidence of a double population. Serum iron 232 μ g, TIBC 267 μ g/100 ml, MCV 105, MCHC 35. Ham's and Crosby's tests were negative and there was no haemoglobinuria. Coombs, direct test, Donath Landsteiner's test and a Wassermann test were all negative.

The diagnosis of aplastic anaemia was confirmed and treatment with metandrenone (Dianabol) 10 mg daily and prednisone 30 mg a day was given. On this treatment the haemoglobin became normal and the thrombocyte count rose to 90 000/ μ l. This remission lasted for about one year. Then the haemoglobin concentration fell again and the patient was readmitted in September 1964.

The haemoglobin now was 8 g/100 ml. The bone marrow was hyperplastic with definite megaloblastic features. The serum folic acid concentration was decreased (0.003 μ g/ml) whereas serum B₁₂ was normal. Folic acid was now given together with Dianabol and prednisone. Haemoglobin values quickly began to rise and six months later the haemoglobin was normal.

One year later the haematological values decreased again and the patient was readmitted in December 1965. Except for fatigue the patient had no complaints. Haemoglobin was 6.5 g/100 ml, thrombocytes 45 000 and leucocytes 3200/ μ l. Reticulocytes were 2.4. The marrow showed activity both in the erythro- and granulocytopenia. The half life of the patient's erythrocytes tagged with chromium 51 was 20 days without evidence of a double population. Ham's test was now positive. Crosby's test questionably positive. There was neither haemoglobinuria nor haemosiderinuria. Donath Landsteiner's test, a Wassermann test and a direct Coombs test were all negative. The patient was discharged on prednisone 30 mg a day, Dianabol and folic acid as before.

Again haemoglobin rose to normal, thrombocytes were 100 000/ μ l, leucocyte count normal. The patient felt completely well and worked full time as a truck driver. The patient was last seen in January 1967. Haemoglobin, thrombocytes and leucocytes were normal; there was no haemoglobinuria and no haemosiderinuria. Ham's test was slightly positive whereas Crosby's test now was negative. The sucrose haemolysis test was positive. Plasma haemoglobin was 5 mg per 100 ml with a moderate increase during sleep. Serum LDH was 23 units (i.e. upper normal limit) with raised values for isoenzymes 1 and 2. The acetylcholine esterase activity in the erythrocytes was normal. The patient was in a hypercoagulable state with increased concentrations of factors II and VII, V and VIII and a slightly accelerated thromboplastin generation. An electroencephalogram was normal. The patient was discharged on prednisone 12.5 mg, Dianabol and folic acid as before and is at present still in complete haematological remission.

Summary. A 36-year-old farmer who developed aplastic anaemia of unknown aetiology. Treatment with prednisone and anabolic hormone resulted in normal haemoglobin and raised the leucocyte and thrombocyte counts. Next he developed secondary folic acid deficiency with depressed blood counts. After folic acid medication the haemoglobin again became normal. At the onset of the disease Ham's test was negative but became positive four years later concomitantly with a relapse of anaemia. Haemoglobinuria and haemosiderinuria have never been found and now—five years after the start of the disease and more than one year after the Ham's test became positive—the patient is completely well with only serological signs of his disease. Treatment with prednisone and anabolic steroid seems to have been beneficial during the whole course of the disease.

Patient no 7 (RH A 22 939)

Ship's captain born in 1917. There was no family history of blood disease and no known exposure to drugs or chemicals. In 1935 the patient had had a single attack

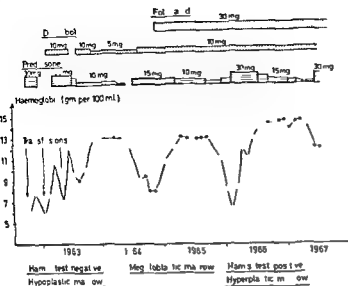


Fig 2 Development of haemoglobin concentration during the course from hypoplastic anaemia to PNH and the treatment given (patient no 6)

of malaria. Except for this and several uncomplicated gonorrhoeal infections he had previously been well.

In 1958 the patient began to suffer from attacks of low back pain accompanied by voiding of dark urine. He felt reasonably well however until the summer of 1964 when he experienced pain in the feet which turned bluish in colour. In December 1964 he was admitted to the local hospital where he was followed during the next years. Physical examination revealed a moderate hepatomegaly. Haemoglobin was 70-80%, leucocytes 10-40 000/ μ l, thrombocytes 500 000-1 300 000/ μ l and reticulocytes 2-10%. In the beginning Coombs direct test was slightly positive but soon became negative. Ham's and Crosby's tests were not performed. The urine was reported to contain haemoglobin on several occasions and periodically the patient was jaundiced with no other signs of liver disease. On the assumption that the anaemia was due to autoimmune haemolysis treatment with prednisone 40-15 mg daily was given. There was no striking response to this treatment and several transfusions of whole blood were given to the patient without any ill effect. In March 1966 an infiltrate in the apex of the right lung was first seen on the chest X-ray. The nature of the infiltrate could not be determined.

In November 1966 the patient was admitted to this department. He complained of fatigue and attacks of slight fever accompanied by low back pain and the voiding of dark urine. The previously mentioned pain in the feet persisted. Physical examination revealed a chronically sick man with the liver extending 5 cm below the right costal margin and the spleen palpable 2 cm below the left costal margin. The skin over the right ankle was bluish in colour. The haemoglobin was 7.9-5.3 g/100 ml, leucocytes 70 000/ μ l (65% granulocytes 2% band forms, 1% metamyelocytes, 1% myelocytes 8% monocytes 5% lymphocytes, 1% normoblasts), thrombocytes 850 000-1 074 000/ μ l (a part giant platelets) and reticulocytes 14.6-29.2%. Bleeding could not be demonstrated. MCV 88, MCHC 30, serum iron 90

μ g/100 ml and TIBC 214 μ g/100 ml. The bone marrow showed marked erythroid and granulopoietic hyperplasia with a normal number of sideroblasts. The Ph chromosome was not present. Plasma haemoglobin was 23 mg/100 ml with accentuated values during sleep. There was haemosiderinuria and occasional haemoglobinuria. Serum haptoglobin 0. Serum LDH was 230 units (ten times the normal value) with an isoenzyme pattern compatible with haemolysis (raised values for isoenzymes 1 and 7). Serum bilirubin 1.4-1.5 mg/100 ml with no other laboratory signs of liver disease. Ham's test was positive. Crosby's negative. The sucrose haemolysis test positive. The acetylcholine esterase activity in the erythrocytes was decreased (138 units). Coombs direct test Donath Landsteiner's test and a Wassermann test were all negative. A haemoglobin electrophoresis was normal. Malaria parasites could not be demonstrated in the blood. Osmotic fragility was slightly increased. Ferokinetic studies with radioiron revealed an effective increased erythropoiesis. An autosurvival study with chromium 51 tagged erythrocytes yielded two slopes: the first indicating a half life of 10.5 days, the other a half life of 21 days. The change in slope of the survival curve coincided with the start of prednisone treatment. Except for the thrombocytosis, coagulation tests were normal. An electroencephalogram was abnormal with sharp-waves/spikes in the left temporal-frontal region. A chest X-ray revealed that the infiltrate in the apex of the right lung persisted. The nature of the infiltrate could still not be determined. Examination of the sputum for tubercle bacilli and malignant cells was negative.

The diagnosis was uncertain. There was good evidence of a PNH defect, but the hepato-splenomegaly and the leuco- and thrombocytosis were not compatible with the conventional picture of PNH. The most likely diagnosis covering these features would be early myelofibrosis and it was therefore assumed that the patient suffered from early myelofibrosis associated with a PNH defect. The possibility that the leuco- and thrombocytosis and the

hepato-splenomegaly could be caused by a malignant tumour in the lung with metastases was not considered very likely in view of the fact that these features had been present at least two years prior to the development of the pulmonary infiltrate. The pain in the feet and the bluish colour of the skin over the ankle were considered to be small thrombotic manifestations of the heavy thrombocytosis.

Treatment with prednisone 40 mg daily and busulfan (Myleran®) was given. Antituberculous treatment was also instituted because of the indeterminate nature of the lung infiltrate. In addition, several transfusions with washed erythrocytes were given. On this treatment the thrombocytes and leucocytes fell to normal values. It was difficult to evaluate the effect of prednisone on the anaemia, but there appeared to be a moderate effect.

The patient was discharged in December 1966 and readmitted in January 1967. During this period palpable lymph nodes had developed on the right side of the neck. A biopsy from these nodes showed metastatic carcinoma. The site of the primary tumour could not be determined with certainty but the histological picture was compatible with a primary bronchogenic carcinoma. This diagnosis found further support from the fact that the infiltrate in the apex of the right lung had grown further and the mediastinum had increased in width. Oedema of the neck developed but regressed somewhat when radiotherapy to the mediastinal mass was given. The patient was discharged in February 1967 on prednisone 15 mg daily. In March 1967 the patient died suddenly at home. No autopsy was performed.

Summary Middle aged man who since 1958 had attacks of low back pain accompanied by the voiding of dark urine. In 1964 hepato spleno megaly, thrombo- and leucocytosis and a haemolytic anaemia which in the beginning was Coombs positive were found. In 1966 good evidence of the PNH defect was observed and the patient was considered to suffer from early myelofibrosis associated with a PNH defect. In 1966 a tumour in the right lung developed, probably a bronchogenic carcinoma. The patient died suddenly in March 1967. No autopsy was performed.

Patient no. 8 (RH A 22 104)

Male civil servant born in 1910. The patient's mother was reported to have had pernicious anaemia, but otherwise no family history of blood diseases could be elicited. There was no known exposure to drugs or chemicals. In 1976 the patient had jaundice, probably due to epidemic hepatitis. In 1933 the patient had haematuria of unexplained aetiology. Otherwise he had previously been well.

During the spring of 1965 the patient felt progressively tired, lost weight and sweated profusely. He was admitted to this department in September 1965. Physical examination revealed considerable enlargement of the

spleen and liver. There was a haemoglobin of 9.5 g/100 ml, leucocytes 10 400-11 100/ μ l and thrombocytes 219 000-280 000/ μ l. A differential count showed 67% granulocytes, 16% band forms, 2% metamyelocytes, 3% myelocytes, 6% eosinophils, 4% lymphocytes, 2% monocytes, 2% myeloblasts and 5% normoblasts. Reticulocytes were 67-76%, serum haptoglobin 48 mg/100 ml. An autosurvival curve with chromium 51 tagged erythrocytes showed a half life of 15 days with no evidence of a double population. No occult bleeding could be demonstrated. Serum iron was 57 μ g, TIBC 347 μ g/100 ml, MCV 102, MCHC 31. A biopsy from the iliac crest revealed a strongly hyperplastic marrow with reticulum cells and many megakaryocytes but no fibrosis. Liver function tests were normal. The basal metabolic rate was 149.

On the diagnosis of early myelofibrosis treatment with prednisone 30 mg a day was initiated and later methylenedione (Dianabol®) 10 mg daily was added to the medication. As haemoglobin fell to 7-8 g/100 ml and the hepato splenomegaly increased X-ray therapy was given to the spleen. 500 r were given but the size of the spleen remained unchanged and the haemoglobin concentration fell further to 6 g/100 ml. The patient was readmitted in February 1967.

There was pronounced haemolysis (reticulocytes 77-35%), a slight leucocytosis and normal thrombocyte counts. During the past month the patient had noticed dark urine on rising in the morning and as haemoglobinuria was now found, tests relevant to PNH were performed. Ham's test was positive, Crossby's test negative, the sucrose haemolysis test was positive. The acetyl choline esterase activity in the erythrocytes was 18.5 units (normal) just after an attack of haemoglobinuria but determination of the enzyme activity performed at the time of no overt haemoglobinuria showed low values (13-14 units). Serum LDH was 257 units with an isoenzyme pattern suggestive of haemolysis (raised values for isoenzymes 1 and 2). Plasma haemoglobin was 15 mg/100 ml. A new autosurvival study with chromium 51 tagged erythrocytes revealed two populations, one with a half life of 7 days, the other with a half life of 16 days. There was a definite accumulation of activity over the spleen. Donath Landsteiner's test, a Wassermann test and Coombs' direct test were all negative. Osmotic fragility was slightly increased. Serum iron was 57 TIBC 269 μ g/100 ml, MCV 113, MCHC 27. There was no stainable iron in the marrow. Coagulation tests revealed hypercoagulability with raised thrombin values and accelerated thromboplastin generation. An electroencephalogram displayed a rather low dominant frequency (8 Hz) but otherwise was normal. The patient was now considered to suffer from concomitant myelofibrosis and PNH. Because of the suspected iron deficiency which might contribute to the pronounced anaemia, oral iron was given for a short period with no discernible effect. Because there was reason to believe that the spleen played a major role in the genesis of the severe haemolytic anaemia, the patient was submitted to splenectomy in March 1967.

The operation was uncomplicated. The spleen weighed 1800 g and showed marked myeloid metaplasia but no

fibrous. The postoperative period was stormy. Immediately after the operation two major complications developed: severe thrombocytosis (thrombocyte counts around 1,500,000/ μ l) and anuria; the latter probably caused by haemoglobinuria precipitated by the infusion of concentrated plasma proteins given in the surgical department. In order to diminish the thrombocytosis and stop the haemolysis with the continuous load of haemoglobin on the kidneys, thrombocytopenesis and exchange transfusions with washed donor erythrocytes were carried out. The thrombocytopenesis was done continuously over a period of three days and the exchange transfusions continuously over a 24-hour period. At the same time peritoneal dialysis was done. Nitrogen mustard was given to restrain platelet production. The result of this intensive treatment was encouraging: the thrombocytosis could decrease to acceptable levels, and the haemolysis was virtually stopped as evidenced by the disappearance of haemoglobin in the plasma and a fall in the reticulocyte count to almost nil. During the following months, however, further complications occurred: high fever probably due to a pulmonary infection, a hepatic disease probably inoculation hepatitis, recurrence of thrombocytosis which necessitated nitrogen mustard and busulfan therapy and deep thromboses of both legs at a time when the platelet count was only moderately elevated and the patient was ambulatory. This in turn was followed by spiking fever which, at least in part, was explained by a small abscess in the abdominal wall. Transfusions with washed erythrocytes had to be given at intervals. Because of these numerous complications an evaluation of the possible effect of the splenectomy cannot be made at the present time. In the months following the operation the only evidence of the PNH defect was a slightly positive sucrose haemolysis test. Haemoglobinuria was constantly present. Ham's and Crosby's tests were negative and the acetylcholine esterase activity in the erythrocytes was normal. It must be borne in mind that a fair part of the erythrocytes at this time were of donor origin. It is also likely that the many transfusions with washed erythrocytes have suppressed the production of erythrocytes with the PNH defect.

Summary Middle aged man with probable myelofibrosis (hepato-splenomegaly with myeloid metaplasia, leuco-erythroblastic haemolytic anaemia, slight leucocytosis, many megakaryocytes and reticulum cells in the marrow and an elevated basal metabolic rate). Eighteen months after the onset of the disease haemoglobinuria appeared and good evidence of the PNH defect was found. After splenectomy numerous complications including thrombocytosis and anuria probably caused by a haemolytic attack occurred.

DISCUSSION

The present series comprises eight patients with the PNH defect seen in this department over a

period of six years. It has been estimated that the incidence of PNH is two per 1 million (6) which would correspond to nine patients in Denmark at the present time. The appearance of eight patients in one medical department (albeit a department with a special interest in haematology) during a relatively short period of time may indicate however that PNH or at least the PNH defect is more common than previously supposed. The reason for this is probably that in recent years PNH has been looked for—and found—in patients who do not fit the classical picture of PNH and certainly not the apparently quite inappropriate name given to the disease. Only one of our patients (no. 1) represented a fairly typical case of PNH whereas three patients (nos. 2, 3 and 4) had long-standing idiopathic pancytopenia and 'haemolytic' anaemia, two patients masqueraded as cases of idiopathic aplastic anaemia (nos. 5 and 6) and two patients were diagnosed as myelofibrosis (nos. 7 and 8).

Diagnosis

Although it is recognized that PNH is sometimes seen in forms which differ from the classical description, most of our patients deviate so much from the original concept of PNH that a consideration of the criteria for the diagnosis is mandatory. Ham's test (the acidified serum test) is recognized as diagnostic of PNH if carried out with adequate controls with unacidified serum, normal erythrocytes and inactivated serum (10). This test was positive in all our patients at one time or another during the course of the disease. The diagnosis does not, however, rest solely on this test but is supported by additional evidence as mentioned below.

The thrombin haemolysis test (Crosby's test) has been very inconsistent in our patients. Positive tests were encountered only in three patients (nos. 3, 5 and 6). The test was often negative in the sense that thrombin did not enhance and sometimes even inhibited haemolysis. Several explanations of this may be imagined. A rather remote possibility would be that patients with a positive Ham's test and a negative Crosby's test might constitute a special group of PNH patients. At the present time there is nothing to support this hypothesis. The reason for the negative outcome of the test in most of our patients is rather a technical one, namely that certain commercial

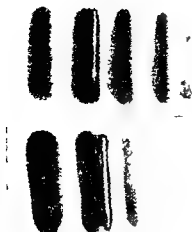


Fig 3 LDH isoenzyme patterns in bone marrow (upper) and in peripheral blood plasma (lower). Anodic isoenzymes ■ the left

brands of thrombin contain additives which inhibit PNH haemolysis (7). A comparison of the brand used by the Statens Seruminstitut Copenhagen where the tests in our patients were performed with other thrombin preparations is at present being made. In view of this and with all the other evidence in favour of the PNH defect in our patients we do not consider that the negative outcome of the thrombin haemolysis test disproves the diagnosis of PNH.

Recently Hartmann and Jenkins have introduced new diagnostic tests for the PNH defect: the sugar water test and the sucrose haemolysis test (21). Whereas the sugar water test varied considerably and was frequently negative the sucrose haemolysis test was positive in all the patients tested also when carried out in isologous compatible serum. Our experience with this new diagnostic test for PNH will be further discussed in a subsequent paper (18). In our opinion the test is of value in the diagnosis of PNH. It appears to be specific and since it is technically easy to perform it will probably become a useful screening test for the PNH defect. Moreover the test may be more sensitive than Ham's test as suggested by the observations in patient no. 8.

As first reported by de Sandre (12) the acetylcholine esterase activity (ACHE) in PNH erythrocytes is low. The aetiological significance of this observation is still unknown (31). Low ACHE is occasionally present in other blood diseases but

only in PNH are low values found consistently. Therefore determination of red cell ACHE is of definite help in establishing the diagnosis of PNH. Whereas in normal blood the highest ACHE activity is found in reticulocyte rich fractions the opposite is true of PNH blood—a finding compatible with the fact that in PNH blood the reticulocyte rich fraction contains the highest proportion of cells susceptible to acid haemolysis (3). However certain experimental models seem to indicate that low ACHE values do not systematically coincide with a positive Ham's test. Consequently determination of enzyme activity may yield information about the cells which is not provided by the acidified serum test (14, 23). In a series of 65 PNH patients Metz (30) found that the most severely ill patients had low values whereas normal values were found in three patients with only mild disease. This is in keeping with our results: the six patients with active disease having had low activities while the patient who only exhibited laboratory evidence of disease (no. 6) had a normal value. The estimation of the severity of the disease from the ACHE activity does however involve one theoretical fallacy: after a significant haemolytic attack the number of PNH cells in the blood may be low and consequently the ACHE activity may be normal. This was observed in one patient (no. 8) who had normal ACHE values just after a haemolytic attack but low activities in periods with no overt haemoglobinuria.

The usual signs indicating intravascular haemolysis present or past i.e. low serum haptoglobin, high plasma haemoglobin, haemosiderinuria and occasional haemoglobinuria were present in varying degrees in our patients. However these signs are not always present even in manifest haemolysis and evaluation of the degree of haemolysis based on these parameters is not always possible. The determining factor among the above mentioned signs is the haptoglobin. The amount of this haemoglobin binding protein is increased during infections in malignancy and during steroid medication (5) and as one or more of these factors were present in many of our patients—and is likely to be in many PNH patients—the applicability of these signs in determining the degree of intravascular haemolysis is therefore correspondingly limited. Similarly other classical signs of haemolysis such as reticulocytosis

Table I Diagnostic criteria of the PNH diagnosis

Pat. no	Ham's test	Crosby's test	Sucrose haemolysis test	Acetylcholine esterase in erythrocytes (normal 10-23.2 units)	Haemoglobinuria and/or haemosiderinuria	Plasma haemoglobin (mg/100 ml)	Serum LDH units (normal <23 units)
1	+	-	+	15.4	+	15	106
2	+	-	+	13.5	+	13	100
3	- → -	+	Not done	Not done	-	Not done	Not done
4	+	-	+	10.3	-	6	100
5	+	+	+	13.5	+	2	98
6	+	(+) → -	-	20	-	4	23
7	+	-	+	13.8	+	16	230
8	+	-	+	13	+	14	257

tosis in the presence of a constant or decreasing haemoglobin concentration and no detectable bleeding may not give a correct estimate of the degree of haemolysis since other factors in the PNH anaemia (e.g. marrow hypoplasia iron deficiency) may influence the picture.

It was therefore felt that other parameters of intravascular haemolysis should also be studied. Determination of plasma lactate dehydrogenase (LDH) activity combined with LDH isoenzyme analysis proved to be of value. These studies were carried out in seven patients. The six patients with active disease all had markedly raised total LDH values (up to 10-15 times normal) whereas the one patient with inactive disease had a high borderline value (patient no. 6). By comparing these values with the respective values for plasma haemoglobin (Table I) it is seen that LDH values are more closely related to the haemolytic activity in most of the patients. In order to determine the origin of the elevated LDH concentrations LDH isoenzyme analysis was performed on erythrocytes and peripheral blood plasma as well as on marrow plasma and marrow nucleated cells. This was done on the assumption that if intramedullary haemolysis—which is sometimes found in PNH patients and is thought to be the source of the markedly elevated LDH values in untreated pernicious anaemia—should contribute to the elevated values this would show up in the isoenzyme pattern. The results are shown in Fig. 3. It is seen that in peripheral plasma isoenzymes 1 and 2 predominate corresponding closely to the erythrocyte pattern whereas in marrow plasma the pattern is dominated by isoenzymes 2, 3 and 4 corresponding to the pattern in nucleated marrow cells. These results of course do not answer the question of

whether or not intramedullary haemolysis is present in PNH but they do seem to indicate that at least the main source of the elevated peripheral plasma values is intravascular haemolysis.

In haematological disorders markedly raised LDH values are found especially in untreated pernicious anaemia whereas most haemolytic anaemias (mainly with extravascular haemolysis) only show moderately elevated values (13). It has even been considered that markedly raised values are indicative of pernicious anaemia but in our experience haemolytic anaemias with intravascular haemolysis including PNH must also be considered when particularly high concentrations are encountered.

The basis for the diagnosis in this series of patients has been discussed so thoroughly because the course of the disease in most of our patients has been rather atypical. If the diagnosis rested solely on a positive Ham's test, doubts as to the significance of this test in our patients could have been raised. However, as demonstrated the diagnosis in each case is supported by other evidence. The diagnostic criteria are summarized in Table I. The exact classification of some of the presented cases may be a matter of opinion but it can hardly be disputed that all of them had the PNH defect.

Relationship to other blood diseases

In the past PNH was considered a primary disease. However, during recent years it has become increasingly evident that the PNH defect sometimes develops in patients suffering from aplastic anaemia. The largest series of patients with aplastic anaemia studied with respect to the

PNH defect was reported by Lewis and Dacie (29) who observed the development of the PNH defect in seven out of 46 patients originally diagnosed as cases of aplastic anaemia. This sequence of events appears to be too frequent to be incidental. The relationship between the two diseases is also supported by the somewhat similar behaviour of the red cells in the two diseases towards complement lysis as demonstrated by Rosse and Dacie (33). Dacie (9) suggests that the PNH defect develops as a complication of aplastic anaemia when haemopoietic regeneration takes place: it is assumed that part of the repopulation is derived from a clone of abnormal stem cells.

Two of our patients (nos 5 and 6) presented as cases of aplastic anaemia of unknown aetiology. The acid haemolysis test, negative at the onset of the disease, became positive after four years. One of the patients (no 5) developed the full picture of PNH six months after the acid haemolysis test was first found positive. In the other patient the PNH defect has till now—more than one year after acid haemolysis was first found—remained a laboratory phenomenon.

During the four years prior to the first demonstration of the PNH defect both patients achieved partial or complete remission on a combined treatment of prednisone and anabolic steroid. The occurrence of acid haemolysis in our patients was heralded by a drop in haemoglobin concentration. The first appearance of a positive Ham's test did not concur with marrow repopulation. However, this is not necessarily an argument against the above mentioned theory of the development of the PNH defect. Ham's test may be a crude test in the sense that it may require a considerable number of abnormal cells in order to yield a positive result, or it may reveal a qualitative red cell defect which is a late event in the development of the PNH syndrome. One way to obtain an earlier diagnosis of the PNH defect might be to apply Ham's test to the youngest erythrocytes which are likely to contain the largest fraction of abnormal cells (29); an even greater refinement may be to study the effect of acidified serum on isotope liberation from radio-iron labeled patient red cells (17). Also the recently developed complement lysis test (33) which measures the complement sensitivity of erythrocytes may prove to be a valuable tool in early diagnosis of PNH

as demonstrated by one of the patients reported by Rosse and Dacie who developed a positive complement lysis test before the acid haemolysis test became positive. Similarly the sucrose haemolysis test may be sensitive enough to aid in early diagnosis. Experience with this test is still limited, but observations like those in our patient no 8 may suggest that it is more sensitive than Ham's test.

It is interesting that the PNH defect was found also in two patients diagnosed as cases of myelofibrosis (patients 7 and 8). Only one case with this combination has previously been reported (11). It is not likely that this is a coincidence. As in aplastic anaemia one might visualize that the strain imposed upon haemopoiesis by the primary disease favours the outgrowth of an abnormal haemopoietic cell clone. Since the basic lesion in myelofibrosis is believed to be a differentiation disturbance of a pluripotential stem cell, one could alternatively speculate that this primary differentiation defect could occasionally manifest itself also in the PNH defect. The tests relevant to PNH were carried out relatively late in the course of the disease and consequently it is difficult to evaluate which of the two diseases is the primary one. In patient 7 there was a history of voiding dark urine six years before signs of myelofibrosis were found. This cannot be further evaluated. In patient 8 frank haemoglobinuria appeared after the diagnosis of myelofibrosis had been established and the appearance of two erythrocyte populations 18 months after the first chromium 51 autosurvival curve had revealed only one population also points to myelofibrosis as the primary disease. None of these facts however prove this sequence. Haemoglobinuria may have gone unnoticed for long periods, or it may have developed late in the disease even if the PNH defect might have been present for a long period of time. Also the appearance of two erythrocyte populations may merely indicate that by that time the PNH cells may have become sufficiently numerous to be revealed in the autosurvival curve. Clearly more observations of combined PNH and myelofibrosis are needed before their relationship can be defined. Finally it should be recognized that although myelofibrosis is the most likely diagnosis in patient 7 the diagnosis is not definitely established. The enlarged liver and spleen during at least three years the

elevated leukocyte count with a shift towards immature cells the thrombocytosis with giant platelets the normoblasts in peripheral blood the pronounced anisocytosis and the moderate poikilocytosis are all in favour of the diagnosis of myelofibrosis. Arguments although not decisive against the diagnosis were that the bone marrow was readily aspirated erythropoiesis was not present in the spleen and was not ineffective as estimated from Fe 59 studies and serum uric acid levels were normal. The balance of the evidence would favour myelofibrosis. In patient 8 the diagnosis of myelofibrosis appears well substantiated spleno-hepatomegaly leucocytosis with metamyelocytes increased numbers of basophilic granulocytes slight thrombocytosis before the operation pronounced poikilocytosis with tear forms normoblasts in peripheral blood increased basal metabolic rate and the slight hyperuricaemia are all in favour of the diagnosis. The biopsy from the iliac crest with reticulum cells and megakaryocytes as the predominant cells and the spleen with myeloid metaplasia with red white eosinophilic and megakaryocyte like cells are also typical of myelofibrosis only fibrosis could not be demonstrated. That fibrosis may be absent in the early stages of myelofibrosis is well known (2).

Among the remaining four patients evidence of a concomitant rare disease was found in patient 4 who developed a positive direct Coombs test and cutaneous manifestations with a histological picture highly suggestive of thrombotic thrombocytopenic purpura (Fig. 1). Also patient 7 had a transient positive Coombs test. These manifestations will be discussed below. In the remaining patients there was no indication of any other blood disease and accordingly their PNH defect must be classified as primary.

COMPLICATIONS OF PNH

Competitive causes of anaemia

The anaemia due to the PNH defect per se is haemolytic. In addition ineffective erythropoiesis and anatomical marrow hypoplasia are sometimes present. To complicate matters even further other mechanisms of anaemia may also be operative. Iron deficiency may develop due to urinary iron loss and folic acid deficiency may appear perhaps as a result of the increased demands during

haemolysis. Occasionally the Coombs test is positive. This could suggest that immune factors may play a role but might just as well reflect the fact simply that a high proportion of the red cells are coated with transferrin.

Iron deficiency develops as a result of the iron lost in the urine as haemosiderin and haemoglobin. The amount of iron lost in PNH has been estimated by a number of authors (reviewed in ref. 20). The results vary considerably probably because it is not always stated whether the lost iron is due to haemosiderinuria haemoglobinuria or both. In these reports the estimated iron loss varies between 1 and 16 mg a day. In a long term study Dac *et al.* (8) found the average iron loss to be 10 mg a day in one of his patients corresponding to an estimated loss of iron of 48 g over a period of 11 years of disease. In a detailed study Hartmann (20) found the average urinary loss due to non haemoglobin iron to be 1.8–7.1 mg/day. To this chronic loss of iron should be added the appreciable amounts of iron which may be lost during occasional major attacks of haemoglobinuria. Thus the development of iron deficiency in PNH patients is readily understandable. Nevertheless iron deficiency in PNH is rather seldom reported (6). The probable reason is that the diagnosis of iron deficiency anaemia superimposed on the PNH anaemia may be difficult. Frequent checks with respect to iron deficiency during the course of the disease should be made. In the careful study by Hartmann *et al.* (20) iron deficiency was found in all seven patients studied.

The usual signs of iron deficiency—low serum iron high TIBC low MCHC and low MCV—do not always give clear cut answers in haemolytic anaemia in which the serum iron tends to be high because of high plasma iron turnover and the MCV tends to be high and the MCHC low because of skipped divisions. The best method of diagnosing iron deficiency is to evaluate the amounts of storable iron in the bone marrow. Five of our eight patients revealed iron deficiency at one time or another. Four of these patients were put on iron medication; the results of this treatment will be reported below.

A megaloblastic component is rarely found in PNH. Few cases have been published in which folic acid deficiency was found (25, 31, 32). One of our patients (no. 6) developed megaloblastic erythropoiesis during the course of his illness at a

Table II *Clinical features*

Pat no	Presentation	Hypercoagulability	Thromboses	Iron deficiency	Abnormal EEG	Effect of prednisone	Coombs test
1	Attacks of abdominal pain	+	Mesenteric (?)	+	Not examined	Not given	-
2	Long standing pancytopenia	-	Cerebral	+	+	-	-
3	Long standing pancytopenia	-	Lower extremity Renal venous	-	Not examined	+	-
4	Long standing pancytopenia TTP (?)	+	Dermal	+	+	+	+
5	Hypoplastic anaemia	+	Mesenteric (?)	+	+	?	-
6	Hypoplastic anaemia	+	-	-	-	+	-
7	Myelofibrosis (?)	+	Lower extremity	-	+	?	+
8	Myelofibrosis	+	Lower extremity	?	+	-	-

time when there was haemolysis but the acid haemolysis test was still negative. Serum B_{12} was normal but serum folic acid was low. His diet seemed adequate and malabsorption could not be demonstrated thus no other reason was found for the folic acid deficiency than the haemolysis with its increased demands. Folic acid administration was followed by a significant rise in haemoglobin concentration.

Coombs positivity. An immunological basis for PNH has never been established. Yet it appears that a positive direct Coombs test is found more often in PNH patients than could be accounted for if the coincidence were fortuitous (6). Two of our patients had a positive direct Coombs test at one time during their illness (patients 4 and 7). The positive Coombs tests were observed at times when the reticulocyte counts were only about 2.1 and 5.0% respectively. Therefore it is not likely that the Coombs positivity was due to transferrin coating of young red cells. In one of them (no 7) the positive Coombs test was transient and the significance uncertain. In the other patient the positive Coombs test was associated with other evidence of immunological disease: presence of the antinuclear factor in the blood and histological features highly suggestive of thrombotic thrombocytopenic purpura (Fig 1). Whereas the diagnosis of TTP seemed certain on histological grounds the lack of leucocytosis and neurological symptoms prompt response of symp-

toms to prednisone and the subsequent benign clinical course are arguments against TTP. To our knowledge a similar histological picture has not been reported in PNH patients.

The occurrence of a positive Coombs test in PNH is usually considered to be a transient episode which is associated with an exacerbation of the anaemia (6). This view is supported by the present observations.

Hypercoagulability—thrombotic complications

Thrombotic manifestations are often encountered in PNH patients. The thromboses may occur everywhere—in the extremities in the brain in the abdomen (6)—the commonest place probably being the mesenteric vessels. It seems reasonable to attribute the numerous attacks of abdominal pain experienced by these patients to small mesenteric thromboses, an assumption sometimes verified at operation (4).

The problem of why thromboses occur so often in a disease in which thrombocytopenia is a frequent feature has not yet been solved but there seems to be general agreement that somehow the thrombotic complications are related to periods of accentuated haemolysis (19). Several authors have demonstrated a state of hypercoagulability even if no agreement has been reached as to which components are responsible for these changes (15).

Coagulation studies were performed in seven

of our patients in three cases tests were made both at the time of moderate haemolysis and during haemolytic attacks with haemoglobinuria. All but one of the seven patients examined had a state of hypercoagulability and the three patients examined during haemolytic attacks displayed increased hypercoagulability during attacks as compared to the more quiescent periods. Indications of thromboses were found in seven patients (see Table II). The detailed results of coagulation studies will be reported separately (1).

EEG changes

Six of our patients had electroencephalograms (EEG) recorded. Varying degrees of abnormality were found in five patients (nos 2, 4, 5, 7 and 8). Four of these patients had spikes universally or in the temporal leads. One patient displayed a rather low dominant frequency. Three patients were examined during sleep. One of these had no changes from the state when she was awake (no 5) whereas the other two (nos 2 and 8) had accentuated abnormalities during sleep.

The significance of these findings is at present completely unresolved. Three possibilities should be evaluated: the changes may be purely incidental; the EEG changes may be secondary to the PNH defect; or the changes may be indications of a cerebral lesion aetiologically related to the PNH erythrocyte defect.

Even if the finding of five abnormal EEGs in six patients is greater than could be expected on a chance basis, the relatively small number of patients precludes conclusions concerning the specificity of the changes. Moreover, alternative explanations of the changes are conceivable in some of the patients: patient 4 may have suffered from thrombotic thrombocytopenic purpura; patient 5 had a history of brain concussion and a hemiparesis like headache; and patient 7 had a malignant tumour which might have metastasized to the brain, even if no clinical symptoms supported this assumption. In the remaining two patients there was no other explanation of the EEG abnormality.

That the EEG changes may be secondary to the PNH defect appears to be a definite possibility in view of the fact that thrombotic manifestations often occur in the cerebral vessels. One of our patients had a history of a cerebral throm-

bosis at the age of 55. It is well possible that in the remaining patients thromboses may have passed unnoticed clinically and merely manifested themselves in abnormal EEGs.

The third possibility, that the EEG changes may be indicative of a primary brain lesion operative in the pathogenesis of PNH, offers ample material for speculations. However, no matter how intriguing such guesswork may be, speculations on the possible significance of the brain and of possible defects in the brain in PNH remain at present futile. From the present data it appears advisable to perform systematic EEG studies in all cases of PNH.

TREATMENT OF PNH

Even if no specific treatment for the PNH defect exists, some aspects of the disease are amenable to therapy. Treatment must be adjusted to the particular problems of the individual patient. Since the problems may vary from time to time, the indications for therapy must constantly be reviewed.

In acute haemolytic crises, transfusions with washed erythrocytes may be life saving. These crises may be life threatening because of the anaemia and—in rare cases—because of acute renal failure due to haemoglobinuria. Renal impairment was seen in patient 8 who developed haemoglobinuria and anuria probably due to a concentrated plasma protein infusion.

Transfusions with whole blood are extremely dangerous in PNH, probably because the transfused blood provides complement. Transfusions to PNH patients must therefore be given as washed erythrocytes. Such transfusions are effective in two ways: they restore the haemoglobin concentration and they tend to diminish the production of defective erythrocytes in the marrow (10). This latter effect was demonstrated in patient 7. When he was transfused with washed erythrocytes, the haemoglobin concentration rose from 7.5 g/100 ml to 11.2 g/100 ml. Concomitantly, the reticulocytes fall from around 500 000/ μ l to 90 000/ μ l.

Transfusions are indicated in severe haemolytic crisis and may also be indicated if during the course of the disease an acceptable haemoglobin level cannot be maintained in other ways. During recent years there has been a tendency to treat even slight anaemia with multiple transfu-

sions. This is hardly justifiable in view of the dangers of multiple transfusions including transfusion haemosiderosis which exists also in PNH in spite of the chronic urinary iron loss. Whereas no stainable iron was found in extrarenal tissues in early reports, the past 10–15 years have brought many reports of stainable iron in extrarenal tissues in multiple transfused cases (20).

As previously mentioned iron deficiency developed in five of our cases. Four of these patients were put on treatment with oral iron during the time of study. One of these (no. 8) was operated shortly after therapy had been initiated and the post-operational period precludes assessment of the value of this treatment. Also patient 4 was difficult to evaluate because prednisone was given at the same time. In patients 1 and 5 the value of the iron treatment can hardly be disputed in both of them the haemoglobin concentration increased by 3–4 g/100 ml over a relatively short period of time. It has been reported that iron medication may provoke haemolytic episodes. In a thoroughly examined group of patients gross haemoglobinuria developed in many of the patients on iron therapy (20). It is difficult to state whether or not the iron therapy has provoked an increased number of haemolytic episodes in our patients. Patient 1 may have experienced more haemolytic attacks while on oral iron but patient 4 has never experienced gross haemoglobinuria and there is nothing to suggest more frequent haemolysis in patient 5.

The megaloblastic anaemia seen in patient 6 at one time during his illness was successfully treated with folic acid. In spite of its rarity the possibility of secondary folic acid deficiency should always be kept in mind.

Treatment with prednisone is usually considered to be of no value in PNH and has even been warned against as thrombotic complications have been attributed to this treatment (10). Nevertheless there are some claims of beneficial results with prednisone therapy (15). In the present series prednisone was given to seven of our eight patients. In two patients (nos. 2 and 8) no effect was seen and in one patient (no. 7) the effect was equivocal. In the remaining four patients however a prednisone effect was seen at one time or another during the course of the illness. In two patients prednisone was effective during the initial period of aplastic anaemia com-

bined with haemolysis. In one of these patients prednisone had no effect when the PNH defect was fully developed (no. 5). The other patient (no. 6) is at present in full haematological remission on a combined treatment of prednisone, anabolic steroid and folic acid. Patient 3 enjoyed an almost normal haemoglobin concentration during one year of prednisone treatment after at least 15 years of pronounced anaemia. During the same period Ham's test turned negative an event which is extremely rare. In the last patient (no. 4) with a positive Coombs test and histological features of thrombotic thrombocytopenic purpura the effect of prednisone could have been on the possible immunological component in the haemolytic anaemia. However in this patient the haemoglobin concentration rose to a level not seen during the past ten years of her illness during which time no evidence of immunological disease had been found. Still the effect of prednisone cannot be evaluated because at the same time iron was given.

In our opinion the possible effect of prednisone in PNH patients cannot be predicted. A trial of prednisone should be made in all PNH patients when anaemia which cannot be explained from iron or folic acid deficiency is present.

A beneficial effect of anabolic steroids in PNH has been reported (20). This treatment was given to four of our patients. In patient 2 the treatment has just been initiated and patient 5 only received this treatment for a relatively short period of time four years before evidence of the PNH defect was found. In patient 8 no effect of the treatment was seen during a six months trial. As previously mentioned, patient 6 is at present in full remission on a combined treatment of prednisone, anabolic steroid and folic acid.

Thus the effect of prednisone and anabolic steroids in the treatment of PNH seems varying and unpredictable. This may simply be due to the capricious course of PNH with incidental improvements which erroneously may be attributed to the treatment given. If one prefers to accept that prednisone and anabolic steroids sometimes do have a therapeutic effect in patients with the PNH defect the unpredictability might be explained in terms of the drug effect on the underlying condition. PNH erythrocytes are believed to develop in several states in which normal erythropoiesis is impaired or haemolysis

exists. If the primary disorder is corrected by corticoid or anabolic steroid treatment anaemia is relieved this might secondarily result in suppression of the abnormal PNH red cell clone with diminished PNH red cell production haemolysis would also decrease.

The use of *anticoagulant therapy* against haemolysis and thrombotic complications has been advocated (6-10). Heparin and dicoumarol have both been used. The reports concerning heparin are conflicting (24). Crosby reports *in vitro* trials showing that in small doses heparin increases haemolysis whereas in large doses haemolysis is diminished. This could not be confirmed in an other study (16). *In vivo* trials have been associated with both increased and decreased haemolysis (16-27). Some are of the opinion that heparin is too dangerous to be used in treatment of PNH (6). Dicoumarol has been advocated in the treatment of PNH both as an inhibitor of haemolysis and for the prevention of thrombotic episodes (6). Whereas the former effect of dicoumarol is not universally accepted most authors agree that dicoumarol has a place in the treatment of PNH patients with thrombotic complications (6-10).

Our experience with dicoumarol treatment is limited to one patient (no. 1) who was put on permanent dicoumarol treatment because of recurrent episodes of abdominal pain. During eight months of treatment however the attacks of pain have been unaffected.

ADDITION IN PROOF

Patient no. 8

The patient recovered from the postoperative complications, and the anaemia was alleviated on treatment with testosterone. Since the alleviation of the anaemia all signs of the PNH defect have disappeared (negative Ham's test, negative sucrose haemolysis test, no haemoglobinuria, normal ACHE activity in the red cells) and a new chromium 51 autotransfusion showed evidence of only one erythrocyte population with a half life of 24 days.

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THE SUCROSE HAEMOLYSIS TEST IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA

Studies on Erythrocytes and Bone Marrow Cells

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Abstract The sucrose haemolysis test has been carried out in seven PNH patients, twenty normal persons and fifty patients with haemolysis not attributable to PNH. It was concluded that the test is valuable in the diagnosis of PNH since it appears to be specific for this disease, seems to be more sensitive than the acidified serum (Hams) test and is quite easy to perform. The significance of slightly positive reactions in three patients with haemolysis other than PNH is discussed. The more amplified test, the sugar water test, has been found to be unreliable. The sucrose haemolysis test has also been carried out on nucleated cells from the bone marrow of seven patients with the PNH defect. These cells were not susceptible to lysis by the sucrose solution.

The diagnosis of paroxysmal nocturnal haemoglobinuria (PNH) is based mainly on the demonstration of intravascular haemolysis, decreased activity of acetylcholine esterase in the red cells and—considered a *sine qua non*—a positive Hams test (the acidified serum test).

Until recently it was generally accepted that PNH was a disease of its own and that a positive Hams test was diagnostic of this disease. Evidence of PNH developing in the course of aplastic anaemia (3) and myelofibrosis (5) has however called for a reconsideration of the concept of PNH. In this period of reevaluation it is desirable to establish the broadest possible basis for the diagnosis and therefore additional diagnostic criteria are of value.

Recently new diagnostic tests for the PNH defect have been developed, viz. the complement lysis test (15) and the sucrose haemolysis test (8). Experience with these tests is still very limited. The purpose of the present work was to study the usefulness of the sucrose haemolysis

test performed with peripheral red cells in the diagnosis of PNH. In addition the sucrose haemolysis test was performed on nucleated bone marrow cells.

METHODS

The sucrose haemolysis test on peripheral red cells

A 10% sucrose solution was prepared by dissolving 10 g of sucrose in 100 ml of distilled water. The pH of this solution is approximately 6.3. Buffering was not used. 0.85 ml of a freshly prepared sucrose solution was mixed with 0.05 ml of autologous serum and 0.1 ml of a 50% suspension of washed erythrocytes from oxalated blood. After incubation for 30 min at 37°C the mixture was centrifuged and the supernatant observed for visible haemolysis. If haemolysis was present the test was repeated with homologous compatible serum instead of autologous serum.

The sugar water test

One ml of oxalated blood was mixed with 9 ml of a freshly prepared sucrose solution made by dissolving 10 g of sucrose in 100 ml of distilled water. The mixture was incubated for 30 min at 37°C, centrifuged and the supernatant observed for visible haemolysis.

The sucrose haemolysis test on marrow nucleated cells

Marrow was aspirated from the sternum, collected in oxalate, transferred to capillary tubes (internal diameter 1.5 mm) and centrifuged at 3000 g for 20 min. In this way the marrow aspirate was separated into four sharply demarcated layers: 1. fatty layer, 2. marrow plasma, 3. buffy coat, containing the nucleated cells and 4. erythrocytes. The sucrose haemolysis test was carried out on the nucleated cells using the same volumes of sugar cells and serum as indicated for the sucrose haemolysis test on peripheral red cells. After centrifugation at 3000 g for 30 min the activity of lactate dehydrogenase (LDH) in the supernatant was determined as an indicator

of lysis. The activity was compared with the activity in a control tube containing isotonic sodium chloride in stead of the sucrose solution.

Lactate dehydrogenase was determined by Laursen's method (12).

Acetylcholine esterase activity in the red cells was measured as indicated by Jørgensen (11) the normal range being 16.0–23.2 $\mu\text{mol/min/g}$ haemoglobin.

MATERIAL

Seven patients with the PNH defect were studied. The histories and the diagnostic criteria of these patients are reported elsewhere (5). In these patients the sugar water test was carried out on erythrocytes and the sucrose haemolysis test on erythrocytes and marrow nucleated cells.

The sucrose haemolysis test was also carried out in 20 apparently healthy persons and in 50 patients with various diseases associated with haemolysis. The criteria for the presence of haemolysis were constant or decreasing haemoglobin concentration associated with increased reticulocyte counts with no evidence of bleeding or red cell mass regeneration. The following diseases with haemolysis were examined (the number of patients in each group is shown after the diagnosis): myelofibrosis 9 autoimmune haemolytic anaemia 4 aquired idiopathic haemolytic anaemia 10 microangiopathic haemolytic anaemia 1 idiopathic pancytopenia 4 aplastic anaemia 2 systemic lupus erythematosus 1 Hodgkin's disease 2 multiple myeloma 1 Waldenström's macroglobulinaemia 1 acute myeloblastic leukaemia 3 chronic myelogenous leukaemia 2 acute lymphoblastic leukaemia 2 and chronic lymphatic leukaemia 8.

RESULTS

The sucrose haemolysis test on peripheral red cells was positive in all seven patients with PNH irrespective of whether autologous or homologous compatible serum was used.

The sugar water test was positive in only three of the seven patients with the PNH defect. Moreover this test was not consistently positive in these three patients.

The 20 normal persons all had negative sucrose haemolysis tests. Of the 50 patients with haemolytic disorders other than PNH 47 had negative sucrose haemolysis tests. Three patients had weakly positive tests.

The first patient was a 61 year old woman with a severe autoimmune haemolytic anaemia. Haemoglobin was 3.9 g/100 ml, reticulocytes 63%, serum bilirubin 5.6 mg/100 ml, plasma haemoglobin 29 mg/100 ml and serum LDH 90 units (normal upper limit 23 units) with an iso-

enzyme pattern compatible with haemolysis. In the serum an agglutinating erythrocyte antibody could be demonstrated (titre by Coombs technique 2048 at 37°C). Haemoglobinuria was present. Ham's and Crosby's tests were negative. The acetylcholine esterase activity in the erythrocytes was normal (17.5 units). The sucrose haemolysis test was weakly positive in autologous serum but negative in homologous compatible serum.

The second patient was a 61 year old man with myelofibrosis. Haemoglobin was 6.4 g/100 ml, reticulocytes 2.5%, serum bilirubin 1.9 mg/100 ml and LDH 33 units with an isoenzyme pattern compatible with haemolysis. Coombs direct test was negative. Neither haemoglobin nor haemosiderin was present in the urine. Ham's and Crosby's tests were negative. The acetylcholine esterase activity in the erythrocytes was low (14.0 units). The sucrose haemolysis test was weakly positive in autologous and homologous compatible serum.

The third patient was a 46 year old woman with myelofibrosis. Haemoglobin was 8.9 g/100 ml, reticulocytes 1.4–2% and serum bilirubin 1.4 mg/100 ml. An autosurvival study with chromium 51 tagged erythrocytes showed a half life of 15 days with no evidence of a double population. Serum LDH was normal. Coombs direct test was negative. There was no haemoglobin or haemosiderin in the urine. Ham's and Crosby's tests were negative. The acetylcholine esterase activity in the erythrocytes was low (15.5 units). The sucrose haemolysis test was weakly positive in autologous and homologous compatible serum.

The sucrose haemolysis test was performed on nucleated bone marrow cells from the seven patients with PNH. No increase in the supernatant LDH activity was found in any case when comparing the sucrose and the saline medium.

DISCUSSION

The theoretical background for the sucrose haemolysis test is not yet fully established. However it has been shown that solutions of low ionic strength such as the sucrose solution used in this test is able to induce complement coating of red cells (13) and it has been demonstrated that the PNH erythrocyte is sensitive to lysis by abnormally small amounts of complement (15).

The initial report on the test (8) demonstrated that it was positive in eight PNH patients whereas it was negative in 25 normal persons in 50 patients with various non haematological disorders and in 75 patients with various haematological diseases. These early results have later been expanded so that at present the test has been performed on more than 200 patients (10) the test still appearing to be specific for PNH.

The present work supports these reports on the value of the sucrose haemolysis test. The test was positive in all the seven PNH patients examined and it was clearly negative in the 20 normal persons and in 47 of the 50 patients with haemolysis which could not be attributed to PNH. The positive outcome of the test in the three patients in whom the diagnosis of PNH could not be definitely established calls for some comment.

In the first of these patients the test was positive in autologous serum but negative in homologous compatible serum. In the initial report (8) it was stated that the test might be positive in acquired immune haemolytic anaemias due to the presence of haemolysins even if this had not been observed. Our patient illustrates this point. The patient had a strong erythrocyte antibody in her serum and whereas the test was positive in autologous serum it was negative when homologous serum was used.

The two patients with myelofibrosis had weakly positive tests both in autologous and homologous serum. In both patients Coombs direct test was negative. Ham's and Crosby's tests were negative and thus the diagnosis of the PNH defect could not be established. Both patients however had low acetylcholine esterase activity in their red cells and it is thus possible that these patients did have the PNH defect to a mild degree if this interpretation is correct it could mean that the sucrose haemolysis test is a more sensitive indicator of the PNH defect than the classical tests. This assumption is supported by the findings in one of our patients with an established diagnosis of PNH. At a time when this patient had been intensively transfused and consequently most of his red cells were donor erythrocytes Ham's and Crosby's tests became negative whereas the sucrose haemolysis test remained weakly positive.

Our experience with the more simplified ver-

sion of the test the sugar water test was not favourable. Only three of the seven PNH patients examined had positive tests and the test was not consistently positive in these three patients. The unfavourable experience with this simple test is in contrast with the results of the initial report (9) in which the test was positive in all of the eight PNH patients examined.

The simple technique of the sucrose haemolysis test makes it applicable in studies of possible lysis of cells other than erythrocytes. In the present work the sucrose haemolysis test was carried out on nucleated bone marrow cells. Even if it is known that PNH haemolysis *in vivo* is due mainly to intravascular destruction the possible contributory role of intramedullary haemolysis (= ineffective erythropoiesis) has not been fully elucidated. Crosby is of the opinion—based on studies of haemoglobin formation—that PNH is similar to pernicious anaemia in this respect i.e. that ineffective erythropoiesis is prominent in the haemolytic mechanism (2). Radioisotope studies using Fe 59 have yielded contradictory results although most investigators agree that erythropoiesis in PNH is effective (1, 4, 9). Other studies using lactate dehydrogenase liberated into marrow plasma as an indicator of lysis also suggest that intramedullary haemolysis is not prominent in PNH (5, 6).

Information on the susceptibility of marrow nucleated cells to *in vitro* haemolysis is scarce. Crosby (2) and Nussey et al. (14) applied the acidified serum test (Ham's test) to bone marrow cells and reported that marrow nucleated cells were destroyed by this procedure. In the present work the sucrose haemolysis test was performed on marrow nucleated cells and liberated LDH used as an indicator of lysis. The use of this enzyme for this purpose seems justified. LDH is known to exist in bone marrow nucleated cells and determination of the enzyme activity can be used as an indicator of intramedullary haemolysis in pernicious anaemia (16). Furthermore when the sucrose haemolysis test is performed on peripheral erythrocytes LDH is liberated together with haemoglobin (7).

Liberation of LDH from the nucleated marrow cells exposed to the sucrose haemolysis system could not be demonstrated in any of the seven PNH patients studied. This may be considered an additional argument against the postulated role

of ineffective haemopoiesis in PNH. Thus there is increasing evidence that erythropoiesis is not ineffective in PNH. If this is accepted one is faced with the problem why PNH reticulocytes are susceptible to lysis whereas the only slightly younger nucleated red cell precursors are not. An obvious difference between the two types of cells is the presence of the nucleus and of RNA synthesis in the nucleated cells. However, whether or how this could confer any protection on the cells must remain a matter of speculation.

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SLEEP RELATED PLASMA HAEMOGLOBIN LEVELS IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA

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Abstract Plasma haemoglobin concentrations have been determined in seven patients with the PNH defect over 24-hour periods, and in four PNH patients during short periods of sleep in daytime. It has been demonstrated that although a rise in plasma haemoglobin during sleep is frequent it is not a constant finding. Contrary to previous reports a splenectomized patient showed characteristic sleep-related rise in plasma haemoglobin during sleep. The observations confirm that plasma haemoglobin levels in PNH patients often vary inversely with the natural variations in plasma cortisol concentrations.

As first demonstrated in the careful studies by Ham (4) the regular periods of increased haemolysis seen in patients with paroxysmal nocturnal haemoglobinuria (PNH) are related to sleep rather than to the night as implied in the name of the disease. Ham studied the diurnal variations in plasma haemoglobin over several days and nights and found increased concentrations of plasma haemoglobin during sleep irrespective of whether the patients slept at night or during the day.

Surprisingly few similar studies have been carried out and at present it is not known whether or not the sleep-associated hyperhaemolysis is a constant finding in PNH patients (2).

The present work was carried out in order to examine the constancy of sleep-related hyperhaemolysis in patients with the PNH defect. It also provided an opportunity to study the effect of splenectomy and corticosteroid medication on PNH hyperhaemolysis.

MATERIAL AND METHODS

Seven patients with the PNH defect were studied. The histories of these patients are reported elsewhere (5) (patients 1, 2, 4, 5, 6, 7 and 8 of that study).

Plasma haemoglobin concentrations were determined every four hours over a 24-hour period. Furthermore plasma haemoglobin concentrations were determined in four patients during short periods of sleep in daytime.

Immediately after venipuncture the oxalated blood was centrifuged at 3000 g for 15 min. Plasma haemoglobin was measured as described by Harboe (6).

RESULTS

The results are shown in Figs 1 and 2. All patients but two showed increased levels of plasma haemoglobin during sleep at night, the maximum level being reached at 12 p.m. or 4 a.m. One patient (no. 6) did not show any significant diurnal variations and one patient (no. 4) had the lowest plasma haemoglobin levels when asleep at night.

In all the four patients examined the plasma haemoglobin level rose markedly during short periods of sleep in daytime with an approximately twofold increase in plasma haemoglobin. As seen in Fig. 2 the increased level of plasma haemoglobin during sleep quickly fell to the pre-sleep level when the patient woke up.

DISCUSSION

This study thus demonstrates that increased levels of plasma haemoglobin during sleep are frequently but not invariably found in patients with the PNH defect. Also the study demonstrates that the changes in plasma haemoglobin concentrations associated with sleep are quickly initiated and terminated.

The two patients who did not show increased levels of plasma haemoglobin during sleep merit further comments. Patient 5 did not show any

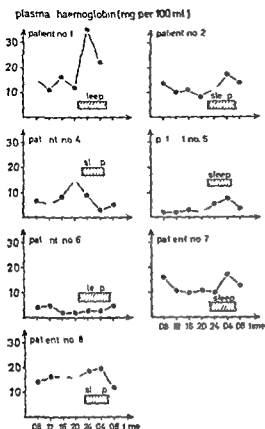


Fig 1 Diurnal variations in plasma haemoglobin

significant diurnal variations which might be explained by the fact that this patient only had a very mild PNH defect. Patient 4 had marked diurnal variations, the highest plasma haemoglobin values being reached when she was awake and ambulatory and the lowest values when she was asleep at night, thus presenting a haemolytic pattern contrary to the pattern usually accepted for PNH haemolysis. Four months prior to this study this patient developed multiple thromboses in the dermal vessels and it must be considered whether part of the haemolysis seen in this patient could be attributed to the mechanism operative in microangiopathic haemolytic anaemia. In this type of haemolytic anaemia haemolysis is strongest when the cardiac output is the greatest (8, 11) which might explain why the plasma haemoglobin concentration was greatest when the patient was awake and ambulatory. However, at the time of the present study the dermal thromboses had vanished and at no time could any bizarre red cells be demonstrated in blood smears, a feature considered a prerequisite for the diag-

nosis of microangiopathic haemolytic anaemia. (1) At present then the only known haemolytic disorder in this patient is PNH which may suggest that even if sleep is usually associated with increased levels of plasma haemoglobin in patients with PNH, this pattern of increased haemolysis is not a constant finding.

The mechanism for the sleep-associated hyperhaemolysis in PNH patients is not known. The *in vitro* test for PNH, the acidified serum test (Ham's test), is based on the fact that PNH erythrocytes haemolyse when exposed to acidified serum and naturally a similar mechanism has been suggested for the *in vivo* haemolysis. It has been suggested that the CO retention seen during sleep might lower the pH in the blood and consequently induce haemolysis. However, a lowering of pH to such a degree as to induce haemolysis has not been demonstrated and it has been shown that hyperhaemolysis is still seen during sleep even when pCO₂ is kept artificially low by having a patient sleep in a respirator.

Ham was of the opinion that even if no significant lowering of pH in the blood could be demonstrated, the local circulation in the spleen might become sufficiently sluggish during sleep to lead to a significantly low pH and thus induce haemolysis. (3) The basis for this assumption was that in one patient the previously found increase of plasma haemoglobin during sleep could no longer be demonstrated after splenectomy. This observation has also been made by others. (7) In the present study, patient 5 had been splenectomized four years previously but still sleep-related

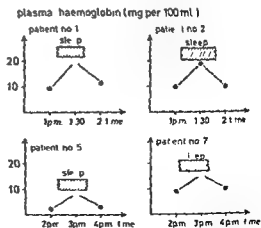


Fig 2 Variations in plasma haemoglobin during short periods of sleep in daytime

hyperhaemolysis was clearly seen as demonstrated in the accompanying figures. It thus appears that the spleen is not alone responsible for the hyperhaemolysis seen during sleep.

Recently it has been noted that the diurnal changes in plasma haemoglobin in PNH patients vary inversely with the diurnal changes in plasma cortisol and it has been suggested that these low plasma cortisol concentrations might have some bearing on the increased haemolysis seen during sleep at night (9). Based on this assumption Morley et al (10) gave glucocorticoids to a patient with PNH and claimed that haemolysis at night was diminished.

The present work supports the observation that the highest levels of plasma haemoglobin are most frequently seen when plasma cortisol is normally lowest. Whether or not the very fast changes in plasma haemoglobin seen during the short periods of sleep in this study can be correlated to low plasma cortisol levels is not known.

In the present study patients 4, 5, 6 and 8 were on prednisone treatment at the time of study. The doses of prednisone given were as follows: patients 4 and 6, 5 mg three times a day; patient 5, 10 mg four times a day; and patient 8, 10 mg three times a day. In the accompanying figures it is seen that this treatment did not significantly suppress the sleep-related hyperhaemolysis.

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COAGULATION AND FIBRINOLYTIC STUDIES IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA

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Abstract Seven patients with paroxysmal nocturnal haemoglobinuria (PNH) have been examined during quiescent haemolysis as well as during haemolytic exacerbations with thrombotic manifestations. In six of the patients hypercoagulability was observed as indicated by short kaolin-cephalin time, accelerated thromboplastin generation screening test and/or increased activity of factors II+VII and VIII. In three cases it was demonstrated that the patient plasma could accelerate the thromboplastin generation of normal plasma. No abnormalities were observed in fibrinolysis nor changes indicative of consumption coagulopathy. The significance of the demonstrated hypercoagulability is unknown. The conclusion of our findings must therefore be limited to the observation that in PNH patients a certain laboratory hypercoagulable state can be demonstrated and that this hypercoagulability increases during the haemolytic crises.

Venous thromboses are a major clinical problem in paroxysmal nocturnal haemoglobinuria (PNH) and the coagulation mechanism in PNH patients has been subjected to a series of investigations. The reports on coagulation have been somewhat conflicting in detail. However, most authors find one or more changes in coagulation indicating a certain activation and hypercoagulability of the clotting mechanism in PNH patients. In this report we present some coagulation and fibrinolytic studies in seven PNH patients.

MATERIAL AND METHODS

Patients

Case reports on the seven patients of this study are presented elsewhere (10). Coagulation studies were performed on the days listed in Tables I-III. All the patients were haemolysing at the time of study, most of them were examined in a period of quiescent haemolysis as well as during haemolytic exacerbations with thrombotic manifestations.

Patient no 1

The first study was carried out during quiescent haemolysis. The next study was performed when the patient woke up at night with abdominal pain (abdominal pain in PNH is usually attributed to small mesenteric thromboses) and signs of increased haemolysis with haemoglobinuria. The next two studies were carried out on the two subsequent days when the increased haemolysis was subsiding.

Patient no 2

Two coagulation studies were performed both during quiescent haemolysis. Prior to these studies the patient had experienced a cerebral episode, probably due to thrombosis, but at the time of study there were no thrombotic manifestations.

Patient no 4

The first study was carried out during increased haemolysis at a time when the patient had multiple dermal thromboses. The next two studies were performed during quiescent haemolysis when there were no signs of thrombosis.

Patient no 5

The first study was carried out during quiescent haemolysis. The next study was performed at a time when the patient had haemoglobinuria and experienced abdominal pain.

Patient no 6

Only one study was made at a time of quiescent haemolysis and no thrombotic manifestations.

Patient no 7

Only one coagulation study was carried out at a time when the patient had pronounced haemolysis and thrombosis in a leg vein.

Patient no 8

Several studies were performed. The first was made during quiescent haemolysis and at a time when there was

Table I Coagulation tests

Test	Patient no 1				Patient no 2		Patient no 4		
	27 9 66	8 10 66	9 10 66	12 10 66	3 7 67	3 5 67	7 6 66	7 7 66	30 11 66
Clotting time } Glasa	8½	—	—	4½	8½	7½	5½	7½	8½
min } Plastic	23	—	—	11½	21½	18½	12	15½	15½
Recalcification time sec	170	—	—	160	190	190	210	170	170
Kaolin-cephalin time sec	41 5/	45/	40/	39/	46 5/	43/	29/	32/	35/
	49 5	54	54	54	48	47	47 5	48	53
Thromboplastin time sec	14/	17 5/	17 3/	16/	15 5/	13 5/	17/	16/	16 5/
	15	17 5	17 5	16	15 5	14 5	17 5	17	18
Factors II + VII	125	85	100	113	100	119	155	130	160
Factor V	—	116	149	150	—	—	220	—	—
Factor VIII	—	138	196	154	—	—	235	—	—
Factor IX	—	—	—	—	—	—	—	—	—
Thromboplastin generation screening test	Accelerated	Accelerated	Accelerated	Slightly accel	Normal	Normal	Accelerated	Accelerated	Accelerated
Thrombin generation test	Normal	—	—	Normal	Normal	Normal	Normal	Normal	Normal
Acceleration of normal thromboplastin generation by patient plasma	—	++	+	0	—	—	0	—	+

0 = no acceleration

-- ++ = acceleration

~ = not investigated

no sign of thrombosis. The following studies were carried out when the patient had undergone splenectomy. Because of heavy thrombocytosis the patient was on anti coagulation treatment as indicated below. The patient developed deep thromboses in both legs.

Reagents

Trisodium citrate 2 H₂O 380 and 313

Di potassium EDTA Solution of 25 mg/100 ml pH 7.40 dried up in the collecting tubes in amounts equalling 0.1 ml/ml of blood

Calcium chloride 1.20 and 1/40 M in distilled water

Owren's buffer 11.75 g of sodium diethylbarbiturate + 14.07 g NaCl in 1570 ml of dist water + 430 ml of 0.1 N HCl pH 7.4 ionic strength 0.15

Phosphate buffers In isotonic saline pH 7.6 and pH 6.1

Thrombin Topostasine® (Roche) dissolved in equal parts of Owren's buffer and glycerol. The stock solution of 100 NIH units/ml was stored at 21°C and diluted with Owren's buffer just before use. The dilution was kept at 4°C and discarded after 4-5 hours.

Protamin sulphate Solution containing 10 mg/ml in water (Leo Pharmaceuticals Denmark)

Thromboplastin Saline extract of human brain prepared for use in the *p*-*p* test (22). Stored at 21°C in small aliquots. After thawing it was kept at 4°C and discarded after 4-5 hours.

Cephalin Human brain cephalin (13) used in optimal concentration diluted with Owren's buffer.

Kaolin Kaolin powder washed in distilled water and

Table II Fibrinolytic tests

Test	Patient no 1				Patient no 2		Patient no 4		
	22 9 66	8 10 66	9 10 66	12 10 66	3 2 67	3 5 67	7 6 66	7 7 66	30 11 66
Fibrinogen g/100 ml	0.3	0.36	0.44	0.38	0.29	0.27	0.30	0.26	0.77
Plasma thrombin time sec	11	12 5/	10 5/	10/	10 5/	11/	12/	10/	11 5/
	11	11	11	10	10	10.5	11	10.5	10.5
Plasma euglobulin clot lysis time min	>300	—	—	120	170	190	150	>240	>300
Fibrin plates mm ³ } Plasma	—	—	—	—	—	—	9	—	0
Euglobulin	—	—	—	—	—	—	90	—	50
Plasminogen	—	—	—	—	—	—	—	—	1.2

* Values of plasma thrombin time in brackets indicate the protamine thrombin time

Patient no 5		Patient no 6	Patient no 7	Patient no 8					Normal range or simultaneous control	
11 11 66	10 1 67	16 1 67	18 11 66	12 67	22 3 67	25 3 67	12 4 67	20 4 67	11 5 67	
8½	—	6½	7½	5½	8½	36	5½	13½	3½	6-12
22½	—	19½	20½	24½	28½	—	27½	26½	37½	15-25
190	—	170	225	170	170	200	150	—	145	165-220
34½	7½	40½	49 5/8	34½	39½	102½	42½	49½	38½	Patient/normal control
48 5/8	46	47 5/8	50 5/8	49	49	54	52	50	45	normal control
17½	19½	19½	19½	17 5/8	24 5/8	37 5/8	18½	22½	17½	Patient/normal control
17 5/8	20 5/8	19	17	16 5/8	15 5/8	16	15 5/8	15	16	normal control
17 3/8	12 3/8	17 5/8	80	100	37	33	76	29	100	80-120
—	145	199	88	—	—	—	—	—	—	80-170
—	263	200	293	—	—	—	—	—	—	60-160
—	—	200	13	—	—	—	—	—	—	60-160
Accelerated	Accelerated	Slightly accelerated	Normal	Accelerated	Slightly accelerated	T _m prolonged	Normal	—	Normal	See text
Accelerated	—	Normal	T _m decreased	Slightly accelerated	Slightly accelerated	T _m decreased	Normal	—	Accelerated	See text
—	+	0	—	—	—	—	—	—	—	

dried at 85°C. Suspensions were prepared in Owren's buffer.

Streptokinase "Streptase" (Behringwerke) dissolved in phosphate buffer pH 7.6. Aliquots of .000 units/ml were stored at -21°C and used just after thawing.

Casein Merck, preparation after Hammarsten.

Fibrinogen Bovine fibrinogen (Povet, Holland) clot ability approx. 56%. The freeze-dried preparation was dissolved in isotonic saline and adjusted with distilled water according to the declaration of each batch to give a final concentration of 1.5% clottable protein and ionic strength 0.15. This stock solution was stored at -71°C and diluted with Owren's buffer just before use.

Adenosine-diphosphate Trisodium salt of adenosine 5-diphosphate (Boehringer & Sohne) dissolved in Owren's buffer 300 µg/ml stored at -21°C. This stock solution was diluted with Owren's buffer before use.

ren's buffer 300 µg/ml stored at -21°C. This stock solution was diluted with Owren's buffer before use.

Tubes, pipettes etc.

Blood collecting tubes Five ml polyethylene tubes with polyethylene cap (Heger Plast, Oslo, Norway).

Tubes for clotting tests and dilutions Polystyrene tubes 70×10/11 mm and 100×15/16 mm with polystyrene cap (Nunc Ltd, Roskilde, Denmark). Glass tubes 80×10/11 mm and 100×15/16 mm. All tubes were used once only.

Petri dishes Disposable sterile acrylic dishes 90×1.5 cm (Nunc Ltd, Roskilde, Denmark).

Pipettes For transfer of plasma after centrifugation.

Patient no 5		Patient no 6	Patient no 7	Patient no 8					Normal range or simultaneous control	
11 11 66	10 1 67	16 1 67	18 11 66	12 67	22 3 67	25 3 67	12 4 67	20 4 67	11 5 67	
0.8	0.46	0.6	0.49	0.54	0.61	0.55	0.53	0.82	0.72	0.2-0.4
10 5/11	10/10.5	11/11	12/10.5	10 5/11	16/11	>180/10	14/11	14/10.5	11/10	Patient/normal control
90	>40	120	>240	>300	(1/10) ⁹	(12/9) ⁹	(12/9 5) ⁹	(14/10)	>300	>120 mean 300 mm
—	0	—	—	—	>40	>240	>300	100	—	
—	49	—	—	—	—	—	0	—	0	0-trace
—	—	152	—	—	—	—	16	—	20	0-50
—	—	—	—	—	—	—	—	—	—	70-120

Table III Tests for platelet function

Test	Patient no 1			Patient no 2			Patient no 4		
	22 9 66	8 10 66	9 10 66	12 10 66	3 2 67	3 5 67	7 6 66	7 7 66	30 11 66
Bleeding time min	2½	—	—	1½	1	2½	1½	7½	3
Right ear	2½	—	—	1½	4	4	1½	2½	1½
Left ear	—	—	—	—	—	—	—	—	—
Platelets $\times 10^9/\mu\text{l}$	226	—	—	227	149	199	141	136	144
Prothrombin consumption index	14	—	—	12	<6	5	<4	<5	<7
Platelet aggr. $\{ 3 \mu\text{g/ml}$	—	—	—	—	13	—	9	—	10
with ADP $\{ 0.3 \mu\text{g/ml}$	—	—	—	—	27	—	15	—	25
Clot retraction 1 h/37 C	—	—	—	—	Normal	—	—	—	Normal
Platelet morphology phase contrast microscopy	—	—	—	—	—	—	Normal	—	Normal
Platelet adhesiveness	—	—	—	—	—	—	—	—	—

disposable polystyrene eye-drop pipettes (Runc Lid Roskilde Denmark) were used. For volumes of 2 ml and less constriction pipettes (Carlsberg pipettes) were used. The pipettes were rinsed in alkaline (pH 9.6) detergent (Tronox[®] Kelanon, Copenhagen Denmark) tapwater and distilled water and finally dried at 37°C.

Cannular Venipuncture cannulas polished inside (Acuterm V2A 1403/12 Ipo). The needles were used once only.

Blood Sampling Technique

Venipuncture was carried out in an antecubital vein or a dorsal hand vein with a minimum of venous stasis. 1–2 ml of blood was discarded and the samplings were performed in the following order and volumes: 2 × 3 ml blood in EDTA glass tubes (80 × 10/11 mm) for platelet count and red blood cell volume; 4 × 1 ml for whole blood clotting times; 4–6 ml in a 100 × 15/16 mm glass tube containing 1 ml of glass beads (diameter 2 mm) for preparation of serum and 5 × 4.5 ml in blood collecting tubes with 0.5 ml of citrate 3.8% in each tube. The citrated blood was immediately cooled in ice water and centrifugation was carried out within 10 min after withdrawal. Platelet poor plasma was obtained after centrifugation for 5 min at 1500 rpm (Christ High Speed Centrifuge). Platelet rich plasma was obtained either after spontaneous sedimentation of the blood for 1–3 h or after centrifugation for 5 min at 1500 rpm (Ecco Superior II B centrifuge). Plasma was transferred to polyethylene tubes with a polystyrene pipette kept at 4°C until it is soon after or immediately frozen at -1°C. Ten samples were analysed within four days after sampling.

Normal control plasma A pooled plasma from 8–10 normal healthy individuals. The plasma was frozen at -21°C in 5 ml aliquots in polystyrene tubes immediately after withdrawal and replaced by a new pool was exchanged at least every third week.

Analytical Methods

Whole blood clotting time One ml of blood was sampled in each of 2 glass tubes (80 × 10/11 mm) and 2 polystyrene tubes (70 × 10/11 mm). The tubes were kept at room temperature and tilted once every 15 sec. The time for solid clotting was recorded on stopwatches and the mean of duplicate determinations calculated. S.D. of single determinations calculated by means of duplicate determinations was for glass tubes $\pm 1/2$ min and for plastic tubes $\pm 2/3$ min.

Kaolin-cephalin time 200 μl platelet poor plasma + 200 μl cephalin + 100 μl kaolin suspension 4% were incubated at 37°C in a 80 × 10/11 mm glass tube. After exactly 3 min 0.05 ml CaCl_2 1/40 M was added and the clotting time in seconds was recorded. The results are means of duplicates. S.D. of single determinations calculated by means of duplicate determinations ± 0.5 sec. For all determinations a simultaneous normal control was run.

Thromboplastin time 200 μl platelet poor plasma + 200 μl thromboplastin prewarmed in a 80 × 10/11 mm glass tube. After exactly 1 min 200 μl CaCl_2 1/40 M was added and the clotting time in seconds recorded. Results are means of duplicates. The test included a simultaneous normal control. S.D. of single determinations ± 0.4 sec.

Plasma thrombin time 400 μl platelet poor plasma incubated for exactly 1 min at 37°C in a 80 × 10/11 mm glass tube. The clotting time after addition of 100 μl thrombin solution 10 N.I.H. units/ml was recorded. Simultaneous normal control. S.D. of single determinations ± 0.4 sec.

Plasma protamine thrombin time This was carried out just as the thrombin time but the thrombin solution contained protamine sulphate 100 $\mu\text{g/ml}$.

Factors II + VII (p and p-test) was determined by the method of Owren and Aas (2). For preparation of standard curves and analytical control prothrombin

Patient no 5	Patient no 6	Patient no 7	Patient no 8							Normal range or simultaneous control
11 11 66	10 1 67	16 1 67	18 11 66	12 67	22 3 67	25 3 67	12 4 67	20 4 67	11 5 67	
9½	—	3½	> 15	1½	½	2½	2½	1½	> 15	1-5
3½	—	4½	> 10	1½	5½	3½	1	—	2	1-5
10	41	184	3380	498	895	1350	1640	531	2100	150-400
11	—	< 5	< 9	< 7	9	—	< 10	< 17	20	0-30
15	—	—	4	—	20	—	7	6	—	7 ± 1.8
15	—	—	6	—	30	—	10	15	—	13 ± 3.0
Slightly reduced	—	—	Normal	—	Slightly reduced	—	Slightly reduced	—	—	—
Asp.	—	—	10-15	—	40-50%	—	20-30%	70-80	90-100	—
cytosis	—	—	abnormal platelets	—	abnormal platelets	—	abnormal platelets	abnormal platelets	abnormal platelets	—
poikilocytosis	—	—	—	—	No spontaneous aggregation	—	No spontaneous aggregation	No spontaneous aggregation	No spontaneous aggregation	—
—	—	—	—	—	—	—	—	—	—	56 ± 10

standard plasmas "Protrol" and "Tegrol" (Pfizer) were used. The results are means of duplicate s.d. of single determinations $\pm 3-4\%$.

Factor V This was estimated by the method of Wolf (7) with modifications using citrated plasma, Owren's buffer as diluent, and human brain thromboplastin. For each determination a new standard curve of our pooled normal plasma was prepared s.d. of single determinations calculated by means of duplicate determinations was 3.5-4 at the 50-100% level.

Factors VIII (AHG) and IX These were estimated by the kaolin-cephalin time system using plasma from patients with known severe haemophilia A and B as substrate plasma (factor level $< 1\%$). 100 μ l substrate plasma + 100 μ l patient plasma dilution + 100 μ l cephalin + 100 μ l kaolin suspension were prewarmed in a glass tube 80/10/11 mm at 37°C. Exactly 3 min later 100 μ l CaCl_2 1/40 M was added and the clotting time in seconds recorded. The patient plasma was tested in 2 or 3 dilutions (usually 1/10-1/80) with Owren's buffer. The results were read in per cent of normal from a standard curve of our normal pooled plasma serially diluted 1/10-1/320 if necessary 1/640. New standard curves were prepared for every 8-10 patients samples. All dilutions were kept on ice and prepared in polystyrene tubes. The s.d. of single determinations varies with the factor concentration. At an AHG and factor IX level of $< 1\%$ the s.d. was ± 0.2 and $\pm 0.3\%$ at the 40-60% level ± 0.8 and $\pm 3.4\%$ at the 80-100% level ± 4.4 and $\pm 5.3\%$ and at the 150-200% level ± 25.5 and 39.4 for AHG and factor IX respectively.

Thromboplastin generation screening test 450 μ l prewarmed Owren's buffer + 500 μ l cephalin + 500 μ l prewarmed CaCl_2 1/40 M were incubated for 1 min at 37°C in a 100 x 15/16 mm glass tube. 50 μ l of platelet poor plasma was added. At exactly one min intervals from the addition, 100 μ l of the incubation mixture was transferred to a series of 80 x 10/11 mm glass tubes

containing 00 μ l CaCl_2 1/40 M and placed in a waterbath of 37°C. Five sec later 100 μ l prewarmed normal substrate plasma diluted 1/2 with Owren's buffer was added to the tube and the clotting time recorded on a separate stopwatch. The thromboplastin generation curve may for example be characterized by the substrate plasma clotting time in sec after 1 and 3 min incubation (1 and 3) the minimal substrate plasma clotting time in sec (t_m) and the incubation time in min at which t_m is reached (T_m). For each test a normal control test with pooled plasma is run. The values of 32 normal individuals during the period of investigation were $t = 116 \pm 10.5$ sec $t = 101 \pm 0.2$ sec $t_m = 15.0 \pm 1.39$ sec and $T_m = 8 \pm 0.8$ min and the mean values of 25 simultaneous controls of our normal plasma pool determined at different days were $t = 110 \pm 13.3$ sec $t = 107 \pm 14.9$ sec $t_m = 15.0 \pm 1.1$ sec and $T_m = 8 \pm 0.6$ min. When a patient sample produced values of 1, or 3 and T_m shorter than 2 x s.d. that of the simultaneous control test we considered the test to be "accelerated".

Demonstration of clot acceleration in patient samples To the incubation mixture of the thromboplastin generation screening test 40 μ l normal platelet poor plasma and 20 μ l platelet poor patient plasma were added simultaneously. The test was then carried out in the usual way. When the curve of the mixture compared with the curve of normal plasma was accelerated as described above the patient plasma was considered to possess clot accelerating properties.

Thrombin generation test This test was carried out with platelet rich fresh plasma according to the description by Ollendorff (21) slightly modified. Owren's buffer was used as a diluent and as fibrinogen the Poviet preparation was used in a concentration of 0.15 g clottable protein per 100 ml in Owren's buffer. Among other things the thrombin curve is characterized by the time of maximal generation t_m and the maximal thrombin concentration.

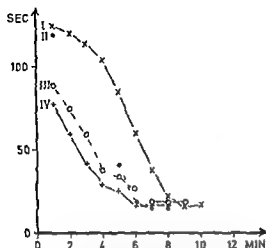


Fig. 1 Thromboplastin generation screening test in patient no. 1 on the 8th (IV) 9th (III) and 17th (II) of October. Curve I is a normal control curve. On the 8th and 9th free haemoglobin in plasma was 8 and 23 mg per 100 ml respectively.

$$\left(\frac{600}{\text{clotting time in seconds}} \right) T_m$$

Values and s.d. in 30 normals were $t_m = 6 \pm 10$ min and $T_m = 44.9 \pm 19.0$. When t_m was 4 min or less the thrombin generation test was considered to be accelerated.

Fibrinogen This was estimated by the method of Jacobsson (14) as modified by Nilsson and Olow (19). The determinations were made in duplicate s.d. of single determinations 0.02 g/100 ml. Antifibrinolytic agents were not added to the blood collecting tubes.

Plasminogen The assay was performed by the method of Alkjaersig et al. (1) as described by Biggs and Macfarlane (3). The trichloroacetic acid soluble fraction was read in a Beckman DU Spectrophotometer at 290 nm. For each test series standards of our normal plasma pool serially diluted with the phosphate buffer pH 7.9 were assayed. The test samples were read on a standard curve prepared for each series of tests and expressed in per cent of the normal s.d. of single determinations 31%.

Fibrinolytic activity This was estimated by the euglobulin clot lysis method as described by Nilsson and Olow (10) and by testing plasma and plasma euglobulin (prepared as described for the above mentioned method) by precipitating plasma with acetic acid at pH 5.4) on fibrin plates (7). Bovine fibrinogen (Povet) was used in a concentration of 0.1% clottable protein in Owren's buffer pH 7.4 ionic strength 0.15. 9 ml of fibrinogen was used for each Petri dish and clotted with 250 μ l of thrombin 10 NIH units per ml in Owren's buffer. Drops of 30 μ l of the test solutions were placed on the plates which were incubated for 18–20 hours at 37°C. The activity was recorded as the product of two perpendicular diameters in mm of the lysed zone (average of 2 determinations). The fibrinolytic measurements were performed within 30 min after withdrawal of the blood.

Bleeding time was determined by the principles of Duke with modifications. In each ear lobe a standardized puncture 4x2 mm was made with a Sera Sharp D (Propper Manufacturing Co. Long Island City NY USA). The bleeding points were wiped gently with filter paper until no more blood was seen. The s.d. of a single determination calculated by means of the duplicate determinations is $\pm 1 \frac{1}{2}$ min.

Prothrombin consumption test This was determined essentially as described by Biggs and Macfarlane (3). Serum was obtained after clotting of venous blood in a glass tube with glass beads as described under blood sampling technique. Citrated serum was used as described by Biggs and Macfarlane. Instead of saline Owren's buffer was used. The fibrinogen was Povet fibrinogen 0.15 g/100 ml in Owren's buffer ionic strength 0.15. The results are means of duplicates.

Platelet aggregation with ADP To 250 μ l platelet rich plasma in glass tubes 80x10/11 mm was added 25 μ l adenosine diphosphate in Owren's buffer 30 and 30 μ g/ml. The tubes were rotated at an angle of 15° in the horizontal plane and the time from addition of ADP until macroscopic aggregates appeared was recorded. The test was carried out at room temperature. The aggregation time was determined with an s.d. of ± 1 sec.

Clot retraction This was determined in 500 μ l of platelet rich plasma + 100 μ l thrombin (10 NIH units per ml) after 1 hour at 37°C. The patient samples were compared with simultaneous normal controls and expressed semiquantitatively as normal, slightly reduced, reduced or absent.

Platelet morphology Platelet rich plasma was observed between an ordinary glass slide and a cover slip in a phase contrast microscope. To evaluate changes in aggregation properties, viscous metamorphosis and other changes during coagulation of the plasma, calcium chloride 1/20 M and platelet rich plasma in the proportion 1 to 2 were mixed on a slide immediately covered and observed in the microscope until the coagulation process was completed.

Platelet adhesiveness was determined by the method of Hellm (17) with only slight modifications. Blood was collected in polyethylene tubes and the sedimentation of the blood dilutions was performed in 75 ml polyethylene tubes. s.d. of a single determination calculated from duplicate determinations was $\pm 4.8\%$.

RESULTS

Table I summarizes the results of the coagulation studies. None of the patients had coagulation defects. The decreased concentration of factors II+VII and the prolonged thromboplastin time in patient 8 on March 22nd and 25th and April 20th were due to oral anticoagulation therapy. On March 25th the patient had also received heparin which was reflected in the prolonged kaolin-cephalin time in the thrombin generation test and in the plasma thrombin time (see Table II).

In contrast several patients showed certain changes which may indicate activation of their clotting system. Thus patients 1 4 5 6 and 8 had short kaolin-cephalin time on one or more occasions. Correspondingly the thromboplastin generation screening test was accelerated in the same patients and the activity of factors II+VII was high in patients 1 4 5 and 6 and of factor VIII in patients 1 4 5 6 and 7. The thrombin generation test was accelerated in patients 5 and 8. In patient 7 the thrombin generation was low on February 2nd and June 11th despite normal factors and high platelet count (see Table III). This was probably due to a qualitative platelet defect as the bleeding time was prolonged and the platelet morphology was slightly abnormal.

No abnormalities were observed in the fibrinolytic measurements shown in Table II. Patient 8 had a high concentration of fibrinogen throughout the period of investigation.

The platelet function tests are shown in Table III. In patient 5 a clear thrombocytopenia was found and a slightly prolonged bleeding time in one ear which can be explained by the low platelet count. In phase contrast microscopy her platelets showed some anisocytosis and poikilocytosis but no definite abnormalities. Patient 7 had severe thrombocytosis, morphologically abnormal platelets and a prolonged bleeding time. ADP induced rapid aggregation in his platelet rich plasma and also the clot retraction was normal. Patient 8 developed severe thrombocytosis during the period of investigation and a large number of his platelets were morphologically abnormal. The aggregation with ADP was normal or slightly reduced and the clot retraction slightly reduced. This last phenomenon and the low platelet adhesiveness to glass beads measured on two occasions may however be a result of the very high platelet number. On one occasion on June 11th his bleeding time was prolonged in one ear.

In all the patients examined during haemolytic crisis evidence of thrombosis was found (patients 1 4 5 7 and 8). Patient 7 had myelofibrosis and thrombocytosis and a possible PNH effect on the coagulation is difficult to evaluate. In patients 1 4 5 and 6 we tried to demonstrate clotting accelerators in the plasma by means of the thromboplastin generation screening test. The results are seen in Table I. Fig. 1 shows the

findings and course in patient 1. It is seen that the patient's plasma induced significant acceleration of the normal generation curve on Oct. 8th which was the first day of an episode with increased haemolysis, a somewhat slighter acceleration on the following day and no definite acceleration five days later.

DISCUSSION

There is general agreement that the thrombotic episodes in PNH patients usually follow periods of accentuated haemolysis. The clotting mechanism of PNH patients has been the subject of several studies. Mainly three mechanisms have been considered to contribute to thrombus formation.

1. An abnormality residing in the platelets which renders them more susceptible to destruction and liberation of thromboplastic material than normal and endows them with increased aggregation properties (7, 17; see also 11). This hypothesis however has not been confirmed by others (cf. 16, 9).

2. Liberation of thromboplastic factors from the abnormal erythrocytes either from the intact abnormal erythrocyte (16) and/or primarily from the reticulocytes (4) or due to release of haemolysate (23).

3. Increased activity of clotting factors, primarily factors VIII (8), V and VII (15).

Decreased clotting activity, probably due to deficiency of factor XI activity was reported by Topp and Hart (25) who concluded that this might be due to consumption by intravascular coagulation. This could be induced by liberation of coagulant material from erythrocytes resulting in thrombin formation which again accelerates haemolysis and creates a vicious circle. With a very sensitive clotting system Newcomb and Gardner (18) demonstrated an accelerated thrombin generation in PNH patients and suggested that the hypercoagulable state probably was related to the formed elements of the blood. With less refined clotting tests changes in PNH plasma could not be detected.

Like Newcomb and Gardner (18) we failed to demonstrate any changes or hypercoagulability in the whole blood clotting time, recalcification time and thromboplastin time. With other methods however some abnormalities could be

demonstrated kaolin-cephalin times were shortened thromboplastin generation was accelerated and in two patients thrombin generation was accelerated. Like others we could also demonstrate increased activity of the clotting factors II + VII, V and VIII in some patients. This might be due to a real increase of concentration but was more likely a result of non specific accelerators in the plasma which exerted their effects in the test systems used. The accelerating effects of PNH plasma on the thromboplastin generation of normal plasma may also be explained in this way.

Our results do not allow any conclusions concerning the mechanism of the clot acceleration in PNH patients. However the demonstration of accelerated thromboplastin generation in patient 1 during haemolysis followed by disappearance of the acceleration after the acute haemolytic attack might point to release of clot accelerating substances during haemolysis. That not only haemoglobin is responsible seems reasonable as the clot acceleration was less on the second day when free haemoglobin in plasma was at its highest. Release of other substances from the lysed erythrocytes for example thromboplastic lipoids (26) has also been proposed (18).

Thrombocytopenia is a frequent finding in PNH patients. It has been explained on the hypothesis that PNH platelets undergo lysis more easily than normal platelets. Against this hypothesis stands the observation that the platelet life span in PNH patients is normal (6, 11).

Lately Rigoczi et al (24) and Brain and Hourihane (5) have drawn attention to the occurrence of haemolysis in the Schwartzman reaction. They also demonstrated in rabbits that intravascular coagulation seems to produce haemolysis which can be prevented by heparin. Such a mechanism might be a possibility in PNH and the thrombocytopenia could be the result of platelet consumption in intravascular coagulation during the crises. If this were the explanation however one would expect to find variations in platelet concentration from time to time. One could also expect to find more or less evidence of clotting factor consumption and/or secondary fibrinolysis in these cases. This was not found in our patients who were repeatedly studied. Only in one patient was a definitely low platelet count observed which was unchanged two months later.

(patient 5). In this patient however it is interesting to note that the thrombin generation and the prothrombin consumption were normal. The thrombin formation was even accelerated despite a very low platelet count which normally results in a low and/or retarded thrombin generation and a poor prothrombin consumption. The findings might indicate that some other platelet like activity was present in the patient's plasma.

The abnormal platelet morphology and platelet function observed in patients 7 and 8 with myelofibrosis were most likely an effect of an abnormal platelet production resulting in the marked thrombocytosis. As mentioned above conclusions concerning the possible PNH effect on coagulation in these patients must be subject to reservations.

The definition of clinical hypercoagulability is a matter of debate and laboratory hypercoagulability is an even more doubtful concept. Up to now there is no proof that laboratory hypercoagulability (shortened clotting time, increased clotting factor concentrations, accelerated thromboplastin and thrombin generation and/or increased platelet clumping tendency) indicates an increased thrombotic risk. Laboratory hypercoagulability may be observed in patients with clinically manifest thrombotic diseases and also in many severely ill patients with a variety of diseases. But it may also be seen in healthy individuals who never had thrombosis. The laboratory hypercoagulability may be the reason why PNH patients develop thromboses or the laboratory findings may be secondary to the formation of thromboses. Furthermore laboratory hypercoagulability might be an *in vitro* phenomenon without any significance *in vivo*. The conclusions of our findings must therefore be limited to the observation that in PNH patients a certain "laboratory hypercoagulable state" can be demonstrated and that this hypercoagulability increases during the haemolytic crises. The studies have not revealed changes in clotting and fibrinolysis indicative of consumption coagulopathy as would be expected during a state of intravascular coagulation.

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Congress Announcements

The Twenty third Annual Symposium on Fundamental Cancer Research GENETIC CONCEPTS AND NEOPLASIA will be held at The Shamrock Hilton Hotel Houston Texas March 5 to 7 1969

Symposium Registration Office address Texas Medical Center Houston Texas 77025 USA

The Fourth International Congress of Nephrology will be held in Stockholm Sweden June 22 to 27 1969

Organizing Committee

President Professor Nils Alwall M D

Vice President and Chairman of the Organizing Committee Professor Bertil Josephson M D

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The Second International Symposium on Gastrointestinal motility will be held in Rome September 10 to 14 1969 The programme will include a limited number of reports followed by discussion and a Round Table The English language will be used throughout Informations from Dr Doc Aldo Tor solti 2 Clinica Medica dell'Universita Viale del Policlinico 00100 Rome Italy

The Seventh International Congress of Diabetes will be held in Buenos Aires August 23 to 28 1970

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